PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 November 2000 (20.11.00)	
International application No. PCT/EP00/02632	**Applicant's or agent's file reference** PU9909
International filing date (day/month/year) 24 March 2000 (24.03.00)	**Priority date** (day/month/year) 24 March 1999 (24.03.99)
Applicant LARSSON, Anders et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 October 2000 (18.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

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Date of mailing (day/month/year)
20 November 2000 (20.11.00)

Applicant's or agent's file reference
PU9909

IMPORTANT NOTIFICATION

International application No.
PCT/EP00/02632

International filing date (day/month/year)
24 March 2000 (24.03.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

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3. Further observations, if necessary:
Please note that the applicant in Box I has assigned all his rights to the applicant indicated in Box II.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
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PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year) 23 April 2001 (23.04.01)	
Applicant's or agent's file reference PU9909	**IMPORTANT NOTIFICATION**
International application No. PCT/EP00/02632	**International filing date** (day/month/year) 24 March 2000 (24.03.00)

1. The following indications appeared on record concerning:

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C L A I M S

1. A method for rendering a surface covered by a polymer material (plastics) more hydrophilic by treatment in a gas plasma of a non-polymerizable gas, **characterized** in that the intensity of the plasma is selected so that the surface becomes permanently more hydrophilic, and with the optional step that the surface subsequently is washed with a solvent selected from water, a water-miscible solvent and mixtures thereof.

2. The method according to claim 1, **characterized** in that the plasma intensity is ≥ 5 W/cm^3/min, in particular with a power ≥ 250 W and a flow ≤ 50 cm^3/min.

3. The method of any one of claims 1-2, **characterized** in that
   (a) the polymer material has been selected among plastics having an immediate water-contact angle $\geq 20°$ and
   (b) the plasma treatment conditions are set so that the immediate water-contact angle after plasma treatment and a subsequent wash with ethanol (70 % w/w) becomes $\leq 30°$, such as $\leq 20°$.

4. The method of any one of claims 1-3, **characterized** in that the polymer material on the surface is selected among
   (a) polymers/copolymers in which the monomers are unsaturated such as (i) alkenes/alkadienes (such as ethen, propen, butadiene and including substituted forms such as vinyl ethers, polyfluorovinyl hydrocarbons (for instance tetrafluoroethylene), and acids, esters, amides, nitriles etc containing one or more alkene groups, for instance various methacryl/acryl compounds; (ii) vinyl aryl compounds in which the vinyl group is bound to aryl (such as mono-, di- and trivinyl benzenes) that optionally may be substituted with for instance lower alkyl groups (C1-6); etc;
   (b) condensation polymers/copolymers in which the monomomers are selected from compounds exhibiting two

Replaced by Claim 34

or more groups selected among amino, hydroxy, carboxy etc groups.

5. The method of any one of claims 1-4, **characterized** in that the plasma is induced by radio- and/or microwaves.

6. The method of any one of claims 1-5, **characterized** in that the plasma gas is oxygen, nitrogen, or a noble gas, such as argon, or a mixture of these gases.

7. The method of any one of claims 1-6, **characterized** in that subsequent to the plasma treatment the surface is derivatized to exhibit positively, negatively and amphoteric groups; hydroxy groups, bioaffinity groups, chelating groups etc.

8. The method of any one of claims 1-7, **characterized** in that at least part of the surface is fabricated into a liquid transportation system of a microfabricated device.

9. A naked plasma treated surface of plastics **characterized** in that the surface has an immediate water-contact angle ≤ 30°, such as ≤ 20°, said water-contact angle being changed less than ± 20% and/or less than ± 5° upon washing with ethanol/water mixture (70% w/w).

10. The naked surface of claim 9, **characterized** in that the surface is made up of a polymer material selected from
(a) polymers/copolymers in which the monomers are unsaturated such as (i) alkenes/alkadienes (such as ethen, propen, butadiene and including substituted forms such as vinyl ethers, polyfluorovinyl hydrocarbons (for instance tetrafluoroethylene), and acids, esters, amides, nitriles etc containing one or more alkene groups, for instance various methacryl/acryl compounds; (ii) vinyl aryl compounds (such as mono-, di- and trivinyl benzenes) that optionally may be substituted with for instance lower alkyl groups (C1-6); etc;

(b) condensation polymers/copolymers in which the monomomers are selected from compounds exhibiting two or more groups selected among amino, hydroxy, carboxy etc groups;

said polymer material optionally being cross-linked and/or optionally being a mixture of two or more kinds of polymers/copolymers.

11. The naked surface of any one of claims 9-10, **characterized** in that the polymer material before having been gas plasma treated exhibits an immediate water-contact angel ≥ 30°.

12. The naked surface of any one of claims 9-11, **characterized** in that at least part of the naked surface is part of a liquid transportation system of a microfabricated device.

13. The use of the surface defined in any one of claims 9-11 and/or obtained by the method defined in any one of claims 1-8 for culturing cells.

14. A kit comprising

(a) a microfabricated device comprising a surface (i) which is manufactured from a synthetic polymer material (plastics) and (ii) on which there are at least one chamber and/or at least one channel, and

(b) a fluorescent substance to be detected in the device, **characterized** in that the plastics has a fluorescence that is non-significant with respect to the fluorescence, in particular with the fluorescence intensity of the plastics being < 50 % of the fluorescent intensity of the substance at the wavelength at which the substance fluoresces.

15. A method for culturing cells that in at least some part of their life cycle require attachment to a substrate surface, **characterized** in that the substrate surface

provides a plastic surface having an immediate water-contact angle ≤ 30°.

16. The method of claim 15, **characterized** in that the plastic surface having an immediate water-contact angle ≤ 30° has been obtained by the gas plasma treatment method defined in claims 1-8 of a surface made of plastics.

17. The method of any one of claims 15-16, with the proviso that for anchorage-dependent cells the conditions have been selected so that at least 30 % of the plated cells adhere to the substrate surface.

18. The method of any one of claims 15-17, **characterized** in that at most 15 % of serum is present in the culture medium.

19. The method of any one of claims 15-18, **characterized** in that culturing is taking place during a time period permitting the number of cells to be at least duplicated.

20. The method of any one of claims 15-19, **characterized** in that the cell culturing is carried out in a chamber of a microfabricated device.

21. A microfabricated device in which the liquid transportation system is formed in/on a polymer material (plastics) that comprises a polymerised aliphatic monomer containing unsaturation.

is attributed to degradation of polymer chains during the plasma treatment.

Similar effects have also been observed for polystyrene. An ESCA-study of a plasma-treated tissue-culture polystyrene surface showed about 35% loss of surface oxygen after water washing (Onyiriuka et al., J. Coll. Interf. Sci. 144(1) (1991) 98). In two other ESCA studies, oxygen-plasma treated polystyrene gave 25% surface oxygen loss after water washing (Callen et al., J. Vac. Sci. Technol. A 13(4) (1991) 2023-2029), (Morra et al., Angew. Macromol. Chem. 189 (3184) (1991) 125-136). A polystyrene surface treated with an oxygen plasma had initially a water-contact angle of 7°, but after a methanol wash the contact angle increased to 64° (Murakami et al., J. Coll. Interf. Sci. 202 (1998) 37-44).

WO 9618498 describes an attempt to produce a permanently hydrophilised surface made of plastics. The method comprises a first step in an inorganic gas plasma in order to introduce charges on the surface and a second step during which a polyionic polymer having the opposite charge is adsorbed to the surface.

EP-A-106,046 describes hydrophilisation of fluorinated polymer surfaces by treatment in a gas plasma in which the main component is a polymerising nitrogen-containing organic compound.

GB 2,061,969 describes the manufacture of hydrophilic and antistatic vinyl chloride polymer by treatment in an inorganic gas plasma. The problem of rendering the plastics permanently hydrophilic is not mentioned.

The electric excitation field applied typically has a frequency in the radiowave or microwave region, i.e. kHz-MHz or GHz respectively. The modification (hydrophilisation) on the polymer surface caused by the plasma will depend mainly on a number of internal plasma parameters such as: type of species present in the plasma, spatial distributions, energy distributions and directional distributions. In turn these parameters depend in a complex way on the external plasma

parameters: reactor geometry, type of excitation, applied power, type of process gas, gas pressure and gas flow rate.

In many applications involving contact between polar liquids and surfaces it is of no big concern whether an introduced hydrophilicity is stable towards washing or not. Particular problems are encountered in case the polymer surface is part of a channel of capillary dimensions, where a high degree of hydrophilicity is necessary if aqueous liquids are to be introduced by self-suction or by centripetal forces. This becomes particularly true in case a repeated contact is to take place reproducibly, in which case an unstable surface modification will be washed away during the first liquid contact. The smaller dimensions of the channel the more severe the problem becomes.

In the context of the invention the expression "plasma treated surface" will, if not otherwise specified, refer to an uncoated naked plasma treated surface, possibly being derivatized to contain separate reactive species firmly bound to the surface.

Cell culturing in microfabricated devices has been described previously in for instance WO 9955827 with priority from April 27, 1998.

Cell aggregates have previously been cultured in vessels with a water contact angle below 30° (JP patent application 19930119579, Derwent abstract accession number 1995-047885).

Adherence of cells to gas plasma treated polytetrafluoro ethylene (PTFE) surfaces with water contact angles 20-45° has been studied (Dekker et al., Clinical Materials 11 (1992) 157-162). Adherence appears to have required abnormally high concentrations of substances promoting adherence (20 % human serum-containing culture medium) compared to the most commonly used 10% or less.

Adherence of CHO cells to surfaces which have been gas plasma hydrophilised in the presence of H_2O-vapour has been studied in order to look for optimal cell cultivation properties of plastics surfaces (Lee et al., Biomaterials

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(**27** pages)
Will also be sent by ordinary mail.

Uppsala, June 13, 2001

Re. International Patent Application PCT/EP00/02632.
Our ref. GY 0018-PCT (formerly PU 9909-PCT)

This is in response to the Written Opinion of April 27, 2001 with a deadline for response of June 3, 2001. A request for extension with 10 days for responding has been requested by fax on June 4, 2001.

NEW CLAIMS
A new set of claims is attached (3x4) plus a set of claims in which the amendments are shown.

For the purpose of simplifying the International Preliminary Examination procedure, claim 1 has been limited to the surface being part of a liquid transportation system in the microformat in which there is a channel, which has a depth ≤ 1000 μm. Support for this limitation is found on pages 12-13, bridging paragraph.

Other amendments are discussed below.

AMENDED DESCRIPTION
On attached pages 2, 3 and 3a (3x3) short references to publications D1, D2, and D3 have been inserted. The pages are attached in triplicate and shall replace original pages 2 and 3. Corresponding pages (1x3) showing the amendments are also attached..

WRITTEN OPINION
Our comments to the Written Opinion are:

I. DEFECTS IN THE APPLICATION.

Item 1.1.1.
The Written Opinion has asked for clarifications regarding the term "surface" in original claims 1, 4, 7-12, 13 and 16. The term has been replaced with "substrate surface" as used in original claim 15. This is in line with the suggestion made in the Written Opinion.

Item 1.1.2.
The Written Opinion suggests that the feature "plastics" within brackets after "polymer material" renders original claims 14 and 21 unclear. Applicants agree and have limited the claims to only comprise substrate surfaces made of plastic material.

Item 1.1.3.
The Written Opinion suggests that the term "non-polymerizable gas" is unclear. The term has been maintained because it is a well-accepted term in the field. See for instance ref D1 page 5, lines 61-67.

Item 1.1.4.
The terminology "conditions are set so that . . ." has been maintained. The inventors have discovered that highly hydrophilic plastic surfaces that are stable against washings with aqueous liquids can be accomplished by treatment in gas plasmas of non-polymerisable gases if the conditions are appropriately set. Permanently hydrophilised surfaces obtained in this way are novel, in particular their use in microfluidic devices in which aqueous liquids are to be transported and for the culturing of cells. In variants that were particularly preferred at the filing date, the conditions for the gas plasma conditions shall be selected within the ranges given in original claim 2. A limitation to claim 2 as suggested in the Written Opinion would exclude Applicants from patent protection for this kind of surfaces when obtained under other conditions. This would unfairly exclude Applicants from obtaining the patent protection they are in their full right to obtain.

Item 1.2.
The Written opinion suggests that the wording "conditions are set so that . . ." render claim 2 unclear. This comment is not understood because claim 2 does not contain this phrase. The ending of original claim 2 ("in particular . . . . ") has in the new set of claims been placed in a separate subclaim (new claim 3).

Item 1.3.
In claim 7 the phrase "positively, negatively and amphoteric groups" has been amended in line with page 11, lines 32-33. The term "bioaffinity groups" is clear for people working in the field, in particular in view of the examples given on page 12, lines 1-8.

The Written Opinion asks for the methods steps used for derivatizing the surface. It should be clear for people in the field that chemical derivatization is contemplated and how to select methods and conditions in order to accomplish a desired group to be introduced.

Item 1.4.
The Written Opinion has asked for a clarification regarding the term "microfabricated" in original claims 8, 12, 14, 20 and 21. All of these claims except claim 21 have been amended as suggested in the Written Opinion. Original claim 21 has been cancelled.

Item 1.5.
The Written Opinion claims that the feature "naked . . surface" is not clear. Original claim 9 has been rephrased to say that the plasma treated surface is in uncoated form.

The Written Opinion also suggests that it should be improper to claim protection for a surface because it is two-dimensional. It is not understood why it is improper to claim protection for this kind of objects. In order to facilitate the handling the case, the term "surface" has been amended to "substrate surface" which is in line with the suggestions for claims 1, 4, 7-12, 13 and 16 made under item 1.1.1 of the Written Opinion.

Item 1.6.
The Written Opinion suggests that the phrase "culturing cells" is not clear. Original claim 13 has been amended to "the culturing of cells". It should be clear from pages 16-17, bridging paragraph what is contemplated. "Culturing of cells" is a well-known concept in the field.

Item 1.7.
The Written Opinion suggests that the term "non-significant" renders claim 14 unclear. The claim has been amended to give relative figures and the wave-length of the fluorescence contemplated. Support is in original claim 14.

Item 1.8.
The Written Opinion suggests that in original claim 15, the cell feature "that in at least some part of their life cycle require attachment to a substrate surface" is not clear. Claim 15 (now claim 17) has been amended to refer to anchorage and non-anchorage dependent cells that for a part of their life cycle require a substrate surface. The expression "Anchorage and non-anchorage dependent cells" has support on page 19, lines 4-5. The critiqued wording is clear for people in the field because it is well-known that there are non-anchorage-dependent cells that for certain parts of their life cycles require the presence of a substrate surface. Compare page 18, lines 31-32.

Item 1.9.
The Written Opinion suggests that original claim 17 is unclear because it doesn't set forth specific conditions for the plasma treatment. Our comments are the same as for item 1.1.1. The claim has been amended to recite that the surface has been selected to enable attachment of at least 30% of the plated cells to the substrate surface. Compare page 17, lines 2-5.

Item 1.10.
The Written Opinion asks for a definition of culture medium and serum. Both terms are well-known in the field. Original claim has been amended to clarify that 15 % of the culture medium is serum (new claim 20).

II. OBSERVATIONS REGARDING NOVELTY AND INVENTIVE STEP IN THE WRITTEN OPINION.

ITEM 2. CLAIMS 1-8.

Item 2.1.
The Written Opinion argues lack of novelty for original claim 1 over D1. It is argued that D1 discloses that gas plasma hydrophilization can result in a permanently more hydrophilic surface.

The feature corresponding to "permanently more hydrophilic" in D1 is not related to the same stability as in the present invention. See page 4, lines 17-37, of D1, where there is a discussion about storage stability. This is different from the present invention for which "permanently more hydrophilic" refers to stability against washing in aqueous media. See our application text pages 5-6, bridging paragraph.

The water contact angles determined in D1 are for porous membranes. It is well-known in the field that when measuring water contact angles for porous surfaces the values obtained will be much lower than for the surface as such. The two reasons for this are

(a) that as soon as the contact angle is below 90 degrees, part of the droplet liquid will penetrate into the pores, reducing the volume in such a way that the macroscopically observed contact angle is lower than the real contact angle at the microscopic surfaces, and

(b) that on a rough surface (such as e.g. a porous membrane), for contact angles below 90 degrees pinning of the of the drop edges on surface protosions will reduce the macroscopically observed contact angle (θ_y) compared to the real microscopic contact angle (θ_w).

The latter can be described by the Wenzel equation $\cos\theta_w = r\cos\theta_y$ where r is the ratio between the actual surface area and the corresponding flat surface area.

Further D1 says nothing about plastic materials.

It follows that both original claim 1 and new claim 1 have novelty over D1.

Item 2.2.
The Written Opinion argues lack of novelty for claim 1 over:
US 4741619: the whole document.
EP 476639: page 4, line 53 – page 5, line 5
page 5, line 10 –lines 27
claims and figures
EP 111795: page 4, line 27 – page 5, line 6
page 6, line 13 – line 21
page 8, line 16 – page 9, line 10

US 4741619: This publication refers to hydrophilizing the surfaces (walls) of microtiter wells. From the text it is clear that any kind of hydrophilization process can be used and a large variety of hydrophilization processes are thus given. The goal is to enable for each well a

liquid meniscus that is perpendicular to a light beam passing through the well. The stability of the created hydrophilicity against washing in aqueous media is not tested.

Applicants have tested nearly all of the methods given in this US patents during their work for finding surfaces that are useful in devices comprising a microscale liquid transportation system and failed to obtain acceptable surfaces. None of the methods tested have provided a surface that is sufficiently stable against repeated contact with aqueous media in order to give reproducible penetration.

It follows that original claim 1 and new claim 1 have novelty over this publication.

EP 476639: This publication concerns atmospheric pressure plasma treatment processes that can be used for a large variety of substrates. Water contact angles are not used as a measure of hydrophilicity. Instead one looks for the tendency for a series of liquids of different surface tension to form drops on the surfaces. The hydrophilicity is obtained in dyne with an increase in dyne corresponding to an increased hydrophilicity. A rough estimation suggests that on surfaces with the water contact angle 20 degrees and lower one could use dyne liquids of up to 72 dynes without drop formation.

On page 12, lines 12-14, it is said that "the hydrophilic nature given by the present invention will not be lost after repeating washing for 20 or more times". This statement says nothing about the washing conditions or to what extent the created hydrophilic nature is retained. The text is silent about the washing liquid being non-aqueous or aqueous.

It follows that original claim 1 and new claim 1 have novelty over this publication.

EP 111 795: This publication concerns hydrophilization of a fabric material (textile) made of synthetic fibres by first adsorbing a chemical agent that will increase the hydrophilicity of the fibre and then stabilising the chemical agent to the fibre by a plasma treatment. This kind of fabric material does not have a surface consisting of plastic material.

It follows that original claim 1 and new claim 1 have novelty over this publication.

Item 2.3.1.
The Written Opinion argues that original claim 2 lacks novelty because page 9, lines 20-27, of D1 discloses the conditions given in claim 2. This is incorrect. Claim 2 and new claim 3 has novelty over D1 for the same reasons as claim 1.

Item 2.3.2.
The Written Opinion argues that original claim 3 lacks novelty over D1 (page 2, line 13). This is incorrect. Original claim 3 and its amended form (new claim 4) have novelty for the same reasons as claim 1. In addition original claim 3 and its amended form (new claim 4) says that the surface obtained after the plasma treatment shall have a water contact angle $\leq 30°$ after a wash in ethanol/water (70%). This is not disclosed in D1.

Item 2.3.3.
The Written Opinion argues that original claim 4 lacks novelty over D1 (page 2, line 14). This is incorrect. Original claim 4 and its amended form (new claim 5) have novelty for the same reasons as claim 1.

Item 2.3.4.
The Written Opinion argues that original claim 5 lacks novelty over D1 (page 9, lines 18 and 67). This is incorrect. Original claim 5 and its amended form (new claim 6) have novelty for the same reasons as claim 1.

Item 2.3.5.
The Written Opinion argues that original claim 6 lacks novelty over D1 (page 2, lines 47 and 48). This is incorrect. Original claim 6 and its amended form (new claim 7) have novelty for the same reasons as claim 1.

Item 2.3.6.
The Written Opinion argues that original claim 7 lacks novelty over D1 (page 8, line 66). This is incorrect. Original claim 7 and its amended form (new claim 8) have novelty for the same reasons as claim 1. In addition new claim 8 and original claim 7 have novelty because page 8, line 66, of D1 does not present any of the groups specified in these claims.

Item 2.3.7.
The Written Opinion argues that original claim 8 lacks novelty over D1 (page 2, lines 3 and 4, and page 3, line 8). This is incorrect. Original claim 8 and its amended form (new claim 9) have novelty for the same reasons as claim 1. For new claim 9 the novelty is further stressed by specifying that the surface contains a liquid transportation system comprising a channel having a depth of ≤ 1000 μm.

ITEM 3. ORIGINAL CLAIM 9-12 (CORRESPOND TO NEW CLAIMS 10-13)

Items 3.1-3.3.
The Written Opinion argues lack of inventive step over D1. The Written Opinion says that the stability defined in our original claim 9 (new claim 10) means that the introduced hydrophilicity measured as water contact angle is changed less than 20 % upon washing in ethanol/water. With respect to D1 the Written Opinion says:

> *D1 highlights the low contact angle (page 9, lines 30 and 31: "Benetzbarkeiten . . . haben sich bemerkenswert verbessert") upon washing with wafer-washing-solution (page 9, line 30: " Wafer Wasch Kuhlmittel . . . Aqua Kool 7X)*

Apparently the Written Opinion has misinterpreted what is done in the experimental part of D1. There is no wash involved in the test protocol given on page 9 of D1. The aqueous liquids are only used as test liquids in order to measure hydrophilicity before and after hydrophilization. The test liquids will in most cases penetrate the porous membrane after the hydrophilization but not before. In other words the hydrophilicity increased as a consequence of the treatment. There is no suggestion that the same liquids are used as washing liquids.

This argument is based on a misunderstanding of the text of D1 and is therefore incorrect. Original claim 9 as well as its amended form (new claim 10) have inventive step.

Items 3.4.1 – 3.4.3.
The argument for lack of inventive step given in the Written Opinion is dependent on the argument given in items 3.1.-3.3 of the Written Opinion. Therefore the Written Opinion is incorrect also with respect to lack of inventive step for original claims 10-12 and their corresponding amended forms (new claims 11-13).

ITEM 4. ORIGINAL CLAIM 13 (CORRESPONDS TO NEW CLAIM 14).
The argument for lack of inventive step given in the Written Opinion is dependent on the argument given in items 3.1.-3.3 of the Written Opinion. Therefore the Written Opinion is incorrect also with respect to lack of inventive step for original claim 13 and its amended form (new claim 14).

ITEM 5. ORIGINAL CLAIM 14 (CORRESPONDS TO NEW CLAIMS 15-16).

Items 5.1-5.2.
The Written Opinion argues lack of novelty for claims 13 (corresponds to new claim 14) over D3 which describes a dark non-fluorescent microtiter plate, the wells of which are used for measuring fluorescence. The amended form of claim 13 (new claim 14) has novelty over D3 since it has been clarified that the presence of one chamber and/or channel means that there is a liquid transportation system comprising a channel having a depth of $\leq$ 1000 µm.

Item 5.3.
The Written Opinion argues lack of novelty of original claim 13 over DE 19739119, which describes a microtiter plate. New claim 14 has novelty for the same reasons as given under items 5.1-5.2.

ITEM 6. ORIGINAL CLAIMS 15 –20 (CORRESPOND TO NEW CLAIMS 17-22).

Item 6.1.
The Written Opinion argues lack of novelty for original claim 15 because D2 on page 52, lines 17-22, and on page 54, lines 1-3, discloses culturing of cells that in at least part of their life cycle require attachment to a substrate surface having an *intermediate* water contact angle which is $\leq 30°$.

Please note that claim 15 does not say *intermediate*.

Page 52, lines 17-22, only discloses "microculture and identification of pathogens". Page 54, lines 1-3, does not disclose anything about culturing of cells. It follows that nothing is said in D2 about selecting the proper surface for culturing of cells that require attachment to a surface for growth and proliferation.

The Written Opinion means that D2 discloses plastic surfaces with a water contact angle which is ≤ 30° on page 19. This is based on a suggestion that page 8 of the present application text gives such values for the plastics mentioned on page 19 of D2. This is incorrect since all the water contact angles given on page 8 of the present application text are ≥ 30°.

Accordingly, original claim 15 and its amended form (claim 17) have novelty over D2.

The fact is that the culturing of cells that in a part of their life cycle require attachment to a surface for growth and proliferation put very strict demands on surfaces. The surfaces shall have the proper hydrophilic/hydrophobic balance and the presence of the proper functional groups, which also shall be essentially non-toxic for the cells.

Items 6.2.1.
The Written Opinion argues that original claim 16 (corresponds to new claim 18) lacks novelty because statements on page 20, lines 8-10, line 14 and lines 31-32, of D1. These passages contain no reference to the culturing of cells.

In fact page 20, lines 8-10, says that cell adsorption is less for hydrophilic surfaces compared to hydrophobic surfaces. If it is presumed that this adsorption is a prerequisite for the culturing of cells that in a part of their life cycle require attachment to a surface, this passage of D1 supports novelty and inventive step over D2 for the invention presented in original claims 15-20 and their corresponding amended forms (claims 17-22).

Item 6.2.2.
The Written Opinion means that original claim 20 (corresponds to new claim 22) lacks novelty because page 5, lines 24 and 28, disclose that the device of D2 comprises a liquid transportation system. This argument is incorrect. Novelty resides in the culturing of cells that in a part of their life cycle require attachment to a surface, and this is not disclosed in D2. See under item 6.1.

Item 6.3.1
The Written Opinion argues lack of novelty over D2 for original claims 17-18. The argument is based on that culturing of cells that adhere to a surface is given on page 20, lines 5 and 6, and page 54, lines 5-12. The written Opinion further suggests that it is an obvious variation to make sure that at least 30 % of the cells adhere to the surface.

This argument is incorrect. Both original claim 17 and new claim 19 have novelty over D2 for the same reason as given in item 6.1.

Item 6.3.2.
The Written Opinion argues lack of inventive step of original claims 18-19 (new claims 20-21) because they represent variations that are obvious for people in the field.

This argument is incorrect because the corresponding independent claim has both novelty and inventive step for the same reasons as given under items 6.1 and 6.2.1 above.

ITEM 7. ORIGINAL CLAIM 21.
Original claim 21 has been cancelled.

CONCLUSION NOVELTY AND INVENTIVE STEP

- From the comments given above it is clear that all claims have novelty.
- None of the cited publications give any solution of the problems relating to the stability of hydrophilized surfaces or to the cell culturing in microfluidic devices. In most of the publications there is not even mentioned liquid transport in microsystem. At least for these reasons all of the present claims also have inventive step.

Based on the comments given above we are expecting a positive International Preliminary Examination Report.

Gyros AB

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G.A. 43194

Enclosures:

- New set of claims (3x4) plus corresponding pages showing the amendments (1x5)
- Retyped pages 2, 3 and 3a (3x3) plus corresponding pages showing the amendments (1x3)
- Copy of General Power of Attorney (43194)
- Form 1037.1 03.99 (only by confirmation letter)

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(57) Abstract

A method for rendering a surface covered by a polymer material (plastics) more hydrophilic by treatment in a gas plasma of a non–polymerizable gas. The method is characterized in that the intensity of the plasma is selected so that the surface becomes permanently more hydrophilic. A naked plasma treated surface of plastics having an immediate water–contact angle ≤ 30°, such as ≤ 20°, said water–contact angle being changed less than ± 20 % and/or less than ± 5° upon washing with ethanol/water mixture (70 % w/w). A kit comprising (a) a microfabricated device comprising a surface (i) which is manufactured from a synthetic polymer material (plastics) and (ii) on which there are at least one chamber and/or at least one channel, and (b) a fluorescent substance to be detected in the device. The kit is characterized in that the plastics has a fluorescence that is non–significant with respect to the fluorescence of the substance at the wavelength at which the substance fluoresces. A microfabricated device having a liquid transportation system which is formed in/on a polymer material (plastics) that is a polymerised aliphatic monomer containing unsaturation.

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SURFACE AND ITS MANUFACTURE AND USES

The present invention concerns a method for enhancing the hydrophilicity of a polymer surface by treatment with a plasma.

A common method for surface modification of plastics is to subject them to various forms of plasma treatment (Chan et al., Surface Science Reports 24 (1996) 1-54; and Garbassi et al, Polymer Surfaces - From Physics to Technology, John Wiley (1998) 238-241). This is done in a plasma reactor, which is a vacuum vessel with a gas at low pressure (typically 10 to 1000 mTorr). When a high frequency electric field is applied over the reactor, a plasma (also called glow discharge) is formed, containing reactive species like ions, free radicals and vacuum-UV photons. These species react with the plastics surface and cause a chemical modification with properties depending on the nature of the gas and on the plasma parameters. Gases like oxygen and argon are typically used for hydrophilisations and adhesion improvement on nonpolar plastics, while vapours of polymerising monomers can be used to apply thin coatings on plastics for a number of different purposes (Yasuda, Plasma Polymerization, Academic Press 1985).

There are a number of publications on treatment of polycarbonate surfaces with oxygen and argon plasmas. A stability study (Morra et al, Angew. Makromol. Chem. 189(3184) (1991) 125-136) showed that much of the hydrophilicity of the treated surfaces was lost after either water extraction or 3 days' dry storage. The hydrophilicity loss after water extraction was due to the formation of low molecular weight water-soluble surface species during the plasma treatment. The storage instability was attributed to rearrangement of the polymer chains in the surface. An ESCA study (Greenwood et al., Macromolecules 30 (1997) 1091-1098) showed that 79% of the oxygen incorporated in the polycarbonate surface by oxygen plasma treatment was removed by washing with a 1:1 cyclohexane/isopropanol mixture. This

is attributed to degradation of polymer chains during the plasma treatment.

Similar effects have also been observed for polystyrene. An ESCA-study of a plasma-treated tissue-culture polystyrene surface showed about 35% loss of surface oxygen after water washing (Onyiriuka et al., J. Coll. Interf. Sci. 144(1) (1991) 98). In two other ESCA studies, oxygen-plasma treated polystyrene gave 25% surface oxygen loss after water washing (Callen et al., J. Vac. Sci. Technol. A 13(4) (1991) 2023-2029), (Morra et al., Angew. Macromol. Chem. 189 (3184) (1991) 125-136). A polystyrene surface treated with an oxygen plasma had initially a water-contact angle of 7°, but after a methanol wash the contact angle increased to 64° (Murakami et al., J. Coll. Interf. Sci. 202 (1998) 37-44).

WO 9618498 describes an attempt to produce a permanently hydrophilised surface made of plastics. The method comprises a first step in an inorganic gas plasma in order to introduce charges on the surface and a second step during which a polyionic polymer having the opposite charge is adsorbed to the surface.

EP-A-106,046 describes hydrophilisation of fluorinated polymer surfaces by treatment in a gas plasma in which the main component is a polymerising nitrogen-containing organic compound.

GB 2,061,969 describes the manufacture of hydrophilic and antistatic vinyl chloride polymer by treatment in an inorganic gas plasma. The problem of rendering the plastics permanently hydrophilic is not mentioned.

The electric excitation field applied typically has a frequency in the radiowave or microwave region, i.e. kHz-MHz or GHz respectively. The modification (hydrophilisation) on the polymer surface caused by the plasma will depend mainly on a number of internal plasma parameters such as: type of species present in the plasma, spatial distributions, energy distributions and directional distributions. In turn these parameters depend in a complex way on the external plasma

parameters: reactor geometry, type of excitation, applied power, type of process gas, gas pressure and gas flow rate.

In many applications involving contact between polar liquids and surfaces it is of no big concern whether an introduced hydrophilicity is stable towards washing or not. Particular problems are encountered in case the polymer surface is part of a channel of capillary dimensions, where a high degree of hydrophilicity is necessary if aqueous liquids are to be introduced by self-suction or by centripetal forces. This becomes particularly true in case a repeated contact is to take place reproducibly, in which case an unstable surface modification will be washed away during the first liquid contact. The smaller dimensions of the channel the more severe the problem becomes.

In the context of the invention the expression "plasma treated surface" will, if not otherwise specified, refer to an uncoated naked plasma treated surface, possibly being derivatized to contain separate reactive species firmly bound to the surface.

Cell culturing in microfabricated devices has been described previously in for instance WO 9955827 with priority from April 27, 1998.

Cell aggregates have previously been cultured in vessels with a water contact angle below 30° (JP patent application 19930119579, Derwent abstract accession number 1995-047885).

Adherence of cells to gas plasma treated polytetrafluoro ethylene (PTFE) surfaces with water contact angles 20-45° has been studied (Dekker et al., Clinical Materials 11 (1992) 157-162). Adherence appears to have required abnormally high concentrations of substances promoting adherence (20 % human serum-containing culture medium) compared to the most commonly used 10% or less.

Adherence of CHO cells to surfaces which have been gas plasma hydrophilised in the presence of H_20-vapour has been studied in order to look for optimal cell cultivation properties of plastics surfaces (Lee et al., Biomaterials

12(5) (1991) 443-448). Poor cell adherence to the most hydrophilic surfaces was found found.

Microfluidic devices in which liquid transportation systems are defined by hydrophilic/hydrophobic barriers have been described previously in for instance WO 9958245 with priority from May 8, 1998.

Experimental results partly corresponding to this invention have been presented at the Second International Symposium on Polymer Surface Modification, New Ark June 1999 (Anders Larsson: Plasma Treated Polycarbonate as Substrate for Culture of Adherent Mammalian Cells).

Objectives of the invention

- A first objective is to provide a gas plasma method for hydrophilisation of polymer surfaces, which enhances the stability of the hydrophilicity introduced.
- A second objective is to provide plasma treated surfaces that are hydrophilic after the treatment and remain so upon repeated wetting/drying, i.e. have an initial hydrophilicity that is not significantly altered in contact with hydrophilic liquids, for instance etanol/water mixtures.
- A third objective is to provide capillary/channel/chamber system, for instance in microfabricated form, having capillaries/channels/chamber, the inner surfaces of which being as defined for the second objective and permitting repeated introduction of aqueous solutions in a reproducible way.
- A fourth objective is to provide liquid transportation systems in which at least a part of the inner surfaces complies with the second objective.
- A fifth objective is to provide plasma treated surfaces that can be used for cell culturing, assay reactions etc.

These objectives are mainly adapted to surfaces that before the plasma treatment have a relatively high immediate water-contact angle, for instance ≥ 20°, such as ≥ 30° or even ≥ 50°. By the term "immediate water-contact angle" is meant that the contact angle is measured on a dry surface before an applied liquid has significantly evaporated. See the experimental part.

The invention

We have now discovered that the objectives given above can be meet, if the polymer surface (plastics surface) is brought into contact with a gas plasma of high intensity (energy input per gas molecule). Our discovery is explainable in terms of two types of polar groups being introduced: (1) Groups retained firmly on the polymer surface and (2) groups allocated to loosely held degradation fragments. The first alternative will result in a stable hydrophilicity. The second alternative will result in a hydrophilicity, which is easily removed by contact with polar liquids, such as aqueous solutions.

Accordingly a first aspect of the invention is a method for rendering a polymer surface (plastics surface) permanently more hydrophilic by contacting the surface with a gas plasma so that firmly bound polar groups are introduced on the surface. Most likely these polar groups are introduced directly on the polymer skeleton constituting the surface, possibly involving cross-linking of the surface layer.

Hydroxy and or amino groups, carboxy groups, ether groups etc and other groups in which a carbon atom binds to a heteroatom selected among oxygen, sulphur, and nitrogen are examples of polar groups that may be introduced. Changes in surface presence of this type of groups may be studied by ESCA (XPS).

The expressions "permanently more hydrophilic" and "stable hydrophilicity" contemplate that the immediate water-contact

angle remains essentially unchanged upon washing with ethanol (70 % w/w, washing procedure as given in the experimental part). This means that that the washing procedure should not be allowed to change the immediate water-contact angle more than ± 20 % and/or more than ± 5°.

The storage stability (in dry form) of the hydrophilised surface should be at least one month with acceptable increases in immediate water-contact water angle not being larger than 10°, preferably not larger than 5°. In case the storage stability in dry form is not acceptable, sufficient storage stability often can be accomplished by storing in aqueous atmosphere or in an aqueous liquid.

The method of the invention may have an optional washing step subsequent to the gas plasma treatment step. This washing procedure means contacting the gas plasma treated surface with an aqueous solution or some other polar liquid to remove loosely held hydrophilic compounds. The washing solution is preferably water, a water-miscible liquid or a mixture of these. Examples of water-miscible liquids are methanol, ethanol, isopropanol, n-propanol, t-butanol, sec-butanol, dimethyl formamide, dimethyl sulphoxide, acetone and other liquid compounds having similar solubilities in water.

The required intensity of the plasma will depend on the variables discussed above. Satisfactory gas plasmas may be found in case the electric excitation power applied is ≥ 250 W with preference for ≥ 300 W, and typically 500±100 W with a gas flow selected in the interval of ≤ 50 cm^3/min, with preference for ≤ 25 cm^3/min. For the plasma intensity, the interval is normally ≥ 5 W/cm^3/min, such as ≥ 10 W/cm^3/min or ≥ 20 W/cm^3/min or even ≥ 35 W/cm^3/min. Normalised values per m^2 electrode area will typically be ≥ 30 W/m^2/cm^3/min, such as ≥ 60 W/m^2/cm^3/min or ≥ 120 W/m^2/cm^3/min or even ≥ 215 W/m^2/cm^3/min. The pressures are typically lower than 100

mTorr, with preference for pressures that are $\leq$ 50 mTorr. These ranges apply for a temperature of 25°C, atmospheric pressure and oxygen. For other gases the values must be multiplied with M_{02}/M_x, where M_{02} and M_x are the molecular weights of oxygen and the other gas, respectively.

The gases used should be non-polymerisable in the type of plasma contemplated. Typical such gases are inorganic. This means that suitable gases are found among oxygen, nitrogen, noble gases (such as helium, neon, argon, krypton, xenon) and mixtures thereof, such as air and mixtures containing other proportions of oxygen and nitrogen. Other potentially useful gases are carbon dioxide, carbon monoxide, water vapour etc that might be used either solely or in combination. By varying the composition of gas the groups inserted onto the surface it is likely that the groups can be varied with respect to kinds and density.

Illustrative examples of polymerisable gases are volatile lower organic molecules such as lower hydrocarbons and vapours of allyl- or acryl monomers, aromatics etc. An inorganic non-polymerisable gas may be present together with a polymerisable organic compound in gas form.

Plasma reactor vessels enabling a sufficiently high power output combined with proper gas flow velocities are commercially available. As stated above the reactor vessels should enable an electric excitation power input for instance in the microwave or radio wave ranges. A suitable plasma reactor is PS0500 (BOC Coating Technology, USA) which permits a radiofrequency (RF) power of 0-500 W and gas flow of 0-100 or 0-1000 standard cm^3/min.

The results of a treatment may depend on the design of the reactor vessel used meaning that the optimal interval to a certain degree will vary from one reactor design to another. The results may also depend on where in the reactor the surface is placed during the treatment.

The immediate water-contact angle of the polymer material (plastics) covering the surface to be gas plasma treated is typically ≥ 20°. The largest advantages with the invention are obtained for polymer materials that provide larger immediate water-contact angles, such as ≥ 30° for instance ≥ 50°. These figures refer to plastic surfaces having been cleaned with respect to water-soluble compounds and low molecular weight compounds (typically ≤ 1 kD). Illustrative examples of how the water-contact angle may vary with polymer are given in table 1.

The inventive method typically results in an increase in the permanent hydrophilicity which corresponds to lowering the immediate water contact angle of the initial plastics surface more than 20 %, such as more than 50 % (after removal of water-soluble compounds, low molecular weight compounds and the like). A large increase in hydrophilicity is more important for hydrophobic than for hydrophilic plastics.

TABLE 1.

Plastic	**Water-contact angle**
PTFE (Teflon)	108°
Silicone rubber (Sylgard 184)	106°
Polypropylene	95°
Polyethylene	94°
Polystyrene	90°
Polycarbonate	78°
PET (polyester)	76°
Styrene-acrylonitrile	73°
PMMA (Plexiglas)	59°

Typically the polymer on the surface has been obtained by polymerisation of monomers comprising unsaturation, such as

in carbon-carbon double bonds or carbon-carbon-triple bonds. The polymer may be a homopolymer or a copolymer.

The monomers may, for instance, be selected from mono-, di and poly/oligo-unsaturated compounds, e.g. vinyl compounds and other compounds containing unsaturation. The monomers may or may not contain halogen substituents, such as fluoro and/or chloro. Illustrative monomers are:

(i) alkenes/alkadienes (such as ethylene, butadiene, propylene and including substituted forms such as vinyl ethers), cycloalkenes, monofluorovinyl and di- and polyfluorovinyl hydrocarbons (for instance tetrafluoroethylene), alkene-containing acids, esters, amides, nitriles etc for instance various methacryl/acryl compounds; and

(ii) vinyl aryl compounds (such as mono-, di- and trivinyl benzenes) that optionally may be substituted with for instance lower alkyl groups (C1-6) etc.

Another type of polymers are condensation polymers in which the monomers are selected from compounds exhibiting two or more groups selected among amino, hydroxy, carboxy etc groups (so called polyfunctional compounds). Particularly emphasised monomers are polyamino monomers, polycarboxy monomers (including corresponding reactive halides, esters and anhydrides), poly hydroxy monomers, amino-carboxy monomers, amino-hydroxy monomers and hydroxy-carboxy monomers, in which poly stands for two, three or more functional groups. Polyfunctional compounds include compounds having a functional group that is reactive twice, for instance carbonic acid or formaldehyde. The polymers contemplated are typically polycarbonates, polyamides, polyamines, polyethers etc. Polyethers include the corresponding silicon analogues, such as silicone rubber.

The polymers may be in cross-linked form.

The polymer on the surface may be a mixture of two or more different polymer(s)/copolymer(s). In this case the water-contact angles and their changes discussed above apply to these mixtures, i.e. the factual surface.

Particularly interesting polymers are those that have a non-significant fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm. By non-significant fluorescence is meant that the fluorescence intensity in the above-given emission wavelength interval should be below 50 % of the fluorescence intensity for a reference plastics (= a polycarbonate of bisphenol A without fluorescent additives). In fact it does not harm in case the fluorescence intensity of the plastics is even lower, such as < 30 % or < 15 %, such as < 5 % or < 1 %, of the fluorescence intensity of the reference plastics. Typical plastics having an acceptable fluorescence are polymers of aliphatic monomers containing polymerisable carbon-carbon double bonds, such as polymers of cykloalkenes (e.g. norbornene and substituterade norbornenes), ethylene, propylenes etc, as well as other non-aromatic polymers of high purity, e.g. certain grades of polymethylmethacrylate.

The requirement for a low fluorescence is of particular importance in case the plastics are to be used for carrying samples in which one or more fluorescent substances are to be detected/ measured. It then becomes important to select plastics with non-significant fluorescence at the wavelength at which a fluorescent substance to be detected/measured fluoresces. In most cases this means that the fluorescence intensity of the plastics at the emission wavelength of the substance should be below 50 % of the fluorescence intensity of the substance. In fact it will not harm in case the fluorescence intensity of the plastics is < 30 %, such as < 15 %, or still lower, such as < 5 % or < 1 %, of the fluorescence intensity of the substance. In case the fluorescence of several substances emitting light at

different wave-lengths are to be measured it will put harsher demands on the plastics, since fluorescence of the plastics then should be non-significant for broader wave-length bands or for several bands.

After the plasma treatment, optionally after a washing procedure to remove loosely held hydrophilic compounds, the surface may be further derivatized to exhibit one or more type of reactive groups, i.e. groups that are able to bind other compounds either via some type of affinity or via covalent linking. Derivatization preferably takes place after the washing step and may be preceded by a coating step, for instance, to provide the surface with a coating carrying additional hydrophilic groups that can be used for derivatization.

By masking certain parts of the surface and leaving other parts unmasked before hydrophilisation, hydrophilic patterns on a hydrophobic surface can be obtained. Alternatively a hydrophobic pattern may be printed on the surface after the hydrophilisation. These techniques may be of value in the manufacture of microfabricated liquid transportation systems. See below.

Various methods for introducing reactive groups on polymers exhibiting hydrophilic groups, such as hydroxy, amino or carboxy etc groups are well known to the averaged skilled artisan in the field. Well known affinity groups are charged groups, and groups exerting affinity via interactions of other types, possibly in combination with charge-charge interactions. Illustrative examples of charged groups are ion-exchanging groups, such as anion and cation exchanging groups, with typical examples being ammonium ions (primary, secondary, tertiary and quaternary ammonium ions), sulphates, sulphonates, phosphates, phosphonates etc. Illustrative examples of other affinity groups are so called bioaffinity

groups including individual members of ligand - receptor pairs, such as antibody - antigen/hapten, complementary nucleic acids, Ig binding proteins - Ig (e.g. protein A or G - IgG), lectins - carbohydrate structures, cells - cell attachment molecules (fibronectin, collagen, RGD-peptides) etc. Included in bioaffinity groups are also semi- and fully synthetic ligands that more or less completely mimics native bioaffinity.

The hydrophilised surface may be part of arrangements of different physical forms. The hydrophilised surface may be the bottoms/walls of microtiter wells and other types of vessels and also the outer surfaces of porous and non-porous particle material. The hydrophilised surface may be part of a less hydrophilic larger surface (e.g. a hydrophobic surface).

The hydrophilised surface may provide a significant part of the liquid contact surfaces in systems intended for transport of aqueous liquids. These systems may have channels that may be of capillary dimensions, for instance with a distance between two opposite walls being ≤ 1000 μm, such as ≤ 100 μm, or even ≤ 10 μm, such as ≤ 1 μm. These systems may also contain one or more chambers connected to the channels and having volumes being ≤ 500 μl, such as ≤ 100 μl and even ≤ 10 μl such as ≤ 1 μl. The depths of the chambers may typically be in the interval ≤ 1000 μm such as ≤ 100 μm such as ≤ 10 μm or even ≤ 1 μm. The lower limit is always significantly greater than the largest of the reagents used. The lower limit is typically in the range 0.1-0.01 μm for devices that are to be delivered in dry form. One or more liquid transportation systems of this type may be placed on a common plate, for instance spinnable, such as a disc of CD-type. In the case of spinnable forms, the liquid may be forced through one or more segments of the transportation system by spinning the disc (centripetal force). In this latter case the liquid transportation systems are placed radially. Other types of

pressure generating systems may also be used for transport of liquid in the liquid transportation systems discussed above.

A device having one or more liquid transportation system comprising channels and chambers with a depth ≤ 1000 µm, such as ≤ 100 µm or even grounder than 10 µm, such as ≤ 1 µm, are further on called a microfabricated device. The chambers/channels are said to be in the microformat. A microfabricated device typically has its channels and chambers in one plane, such as in the surface of a plate, for instance on a disc. The plate may be circular, oval, rectangular or of any other 2D geometric form.

The channels and/or chambers are defined by liquid barriers, which are to guide a liquid flow. The liquid barriers can be in form of physical walls, bottoms and tops. Walls in form of hydrophobic barriers for guiding aqueous liquids and in form of hydrophilic barriers for guiding non-polar liquids have been suggested (WO 9955827 with priority from April 27, 1998). By covering a surface (I) to be hydrophilised with a mask leaving a pattern of communicating lines and dots unmasked and hydrophilise, for instance according to the present invention, the surface (I) will exhibit a hydrophilic pattern. When placing a hydrophobic surface (II) (cover or top) against the hydrophilic pattern and leave a capillary slot between the surfaces, a liquid transportation system will be obtained. Surface (II) may also have a hydrophilic pattern matching the hydrophilic pattern of surface (I). As discussed above the hydrophilic pattern may also be obtained by hydrophilising the full surface and then print the desired hydrophobic pattern thereon. The top/cover will prevent evaporation of liquid. It may have minor parts/dots in form of through-passing holes intended for addition/removal of liquids.

An advantageous way of attaching the top/cover to the hydrophobic surface is by thermogluing as described in SE application 0000300-4, filed on January 30, 2000 (which is hereby incorporated by reference). The top/cover should also

allow for gas exchange between the cultivation chamber and ambient atmosphere.

Liquid transportation systems of he type referred to above may also contain valves, pumps, filters and the like.

The surface may be used for performing chemical reactions of inorganic and/or organic/biochemical nature. The surface may be used as carrier matrix in chromatography, for cell culture, for solid phase chemical synthesis of oligo/polypeptides, oligo/ polynucleotides, other organic polymers and other organic compounds. Illustrative examples of reactions to be run on the surface of the invention are conventional chemical reactions or reactions that are based on affinity involving recognition through geometric fitness and interactions based on hydrogen-bonding, van-der Waals bonding, dipole-dipole interaction, charge-dipole interaction, charge-charge interaction etc. Vessels having interior surfaces being treated according to the invention may be used for storage of various types of organic and inorganic chemicals and/or liquids. For cell culturing, further details are given below.

Advantageous further developments of surfaces obtained by the present invention are described in International Patent Application PCT/EP99/10347 (which is hereby incorporated by reference). In these developments the inventive plasma hydrophilization has been applied to part of or to the complete liquid transportation system. After hydrophilization, polyethylene imine to which monomethoxy polyethylene glycol chains are bound is adsorbed to the treated surface. Preferred designs of liquid transportation systems are also described.

A second aspect of the invention is a naked plasma treated polymer surface (plastics surface) permanently hydrophilised

as defined above and complying with anyone of the other above-mentioned features, either alone or in combination.

A third aspect of the invention is the various uses discussed above or below of surfaces obtained in accordance with the invention and/or having any of the features discussed herein and achievable through the inventive hydrophilisation method.

Kit containing a microfabricated device

A fourth aspect is a kit containing

(a) a microfabricated device comprising a liquid transportation system in which there are at least one chamber and/or at least one channel the walls of which comprises a synthetic polymer (plastics), and

(b) a fluorescent substance to be detected in the device,

The kit is characterized in that the synthetic polymer material has a fluorescence that is non-significant in the same sense as discussed previously in this specification. The surfaces in the channels and chambers of the microfabricated device may wholly or partly be hydrophilised, for instance by gas plasma treatment, preferably according to the method described herein. With respect to the chemical composition of the plastics of the walls (surfaces) and its physical parameters, the same material with the same preferences as given above may be used.

Alternative hydrophilisation protocols are treatment with oxidating acids and with UV-oxidations, corona treatment, grafting and conventional coating with a polymer providing an increased number of polar groups, etc on the liquid contact surface of the material. The polar groups referred to are for instance hydroxy, amino, carboxy, amido, polethylene oxide etc.

Illustrative examples of fluorescent substances are fluorophores of organic or inorganic origin. In the former case they often have a low molecular weight (typically < 1

kD). Important fluorophors have distinct emission wavelengths with distinct maxima in the interval 400-750 nm, with preference for the interval 480-670 nm. Fluorescein, phycocyanines that may be native or chemically modified, rhodamine, Texas Red, fluorescent rare earth chelates (in particular europium and terbium), cadmium selenide nanoparticles etc are typical examples. When in use the fluorophores may be in conjugate form, i.e. covalently attached to a reagent used. In this form the fluorescent substance typically has a molecular weight > 1 kD. The kit, in particular the chambers and the channels combined with the fluorescent substance, may be used for running chemical reactions, assays, separations, cell culturing and the like as described elsewhere in this specification.

A microfabricated device.

A fifth inventive aspect is a microfabricated device as defined above, in which the liquid transportation system is formed in/on a polymer material (plastics) in which the plastics essentially consist of one or more polymers obtained by polymerising one or more aliphatic monomers of the kind defined above. In this aspect the device may have one or more of the features of the other inventive aspects described in this specification. The same uses apply.

Cell culturing

A sixth inventive aspect is a method for culturing cells. The term culturing of cells as used herein includes monolayer culture, suspension culture etc, and excludes culturing of cell aggregates, tissues, biopsies etc. Cell culturing as contemplated herein encompasses inherent normal cell culturing practice, for instance

(a) The number of cells should be at least duplicated or at least triplicated during the cultivation period.

(b) Cultivation of anchorage-dependent cells, which represent an inhomogeneous phenotypic population,

should take place under a low selection pressure for cell adherence to the surfaces used. This implies that for anchorage dependent cells the cell surface should be selected so that at least 30 % of the plated cells should adhere to the substrate surface. More preferably, this adherence percentage should be above 50 % or higher, such as at least 90 %.

(c) In order to promote interaction between cells and substrate surfaces, cell adhesions factors are typically present in the culture medium. For mammalian adherent cells the culture medium typically contain up to 15 % (w/w) serum according to well-established practice.

Culturing of cells, in particular anchorage-dependent cells, have previously been carried out in the presence of substrate surfaces made of plastics. The immediate water-contact angle has been 40°-60°. For cell culturing in microfabricated devices, there will be problems with liquid fluidics with this relatively low hydrophilicity.

It has now been found that culturing of various kinds of cells can be carried out in contact with superhydrophilic substrate surfaces made of plastics having an immediate water-contact angle that is significantly lower than 40°-60°. When applied to microfabricated devices this discovery will improve the situation with respect to liquid fluidics. The sixth aspect thus is characterized in that the cell culturing takes places in the presence of a surface made of plastics providing an immediate water-contact angle ≤ 30°, such as ≤ 20°. The surfaces are primarily provided on the inner walls of the culture vessel, but may also be provided by e.g. particles suspended in the vessel.

The immediate water-contact angle refers to hydrophilicities that preferably are stable against repeated washing as described above.

Surfaces that have been hydrophilised by gas plasma treatment as described in this specification are preferred.

The preferred plasmas contain one or more gases that solely or in combination can introduce the mix of charged/polar groups discussed below. Among the gases tested, mixtures of oxygen and nitrogen are most preferred, with the individual gases being less preferred and with argon being least preferred. One can envisage that gases, such as sulphur dioxide and diphosphorous pentoxide, might be beneficial to use together with oxygen and/or nitrogen, if disregarding the handling problems they might give.

The most important factor for successful culture and behaviour of anchorage-dependent cells is the surface on which the cells grow. If this type of cells are plated onto a surface to which they cannot adhere or adhere poorly they will not grow. Cell function requires dynamic interactions between the cell and its substratum. These interactions occur at specialised contact sites where transmembrane proteins (integrins; Hynes RO, Integrins: versatility, modulation and signalling in cell adhesion. Cell 69: 11-25, 1992) link the interior of the cell with the external substratum. Surface functional groups and their charge character as well as hydrophilicity/hydrophobicity and surface free energy are important factors for cell behaviour (Lee JH et al., Biomaterials 18:351-358. 1997). Amine (ammonium), amide, hydroxyl, carboxyl (carboxylate) and sulphonyl (sulphonate) and sulphate groups at a suitable density are considered as sites for electrostatic interaction with the cell surface or attachment proteins and as mimicking adhesive contacts on extracellular matrix components (Maroudas, J. Theor. Biol. 49 (1975) 417-442; Lee et al., Biomaterials 15 (1994) 704-711; and Lee et al., Biomaterials 18 (1997) 351-358).

Non-anchorage dependent cells often require substrate surfaces during a certain part of their life cycle.

Typically the density of charged groups should be above 1-2 group per $Å^2$. As a thumb of rule smaller cells, such as HeLa cells (10-20 μm), require higher densities than larger cells, such as fibroblasts (about 30x100 μm), lower densities. The

optimal values vary among cell types and may be determined as known in the art.

Cell culturing according to this aspect of the invention applies to a wide variety of cells. The cells may be anchorage- or non-anchorage-dependent. They may be of normal or tumour origin and they may be genetically manipulated in culture. They may be derived from mammals, bacteria, fungi (yeast), plants, fish, birds, amphibians, reptiles, etc. With respect to mammalian cells they may derive from any tissue, e.g. epithelial, endothelial, fibroblast, muscle, nerve, pigment, hematopoetic and germ cells.

For each respective kind of cell, the rules for selecting conditions and protocols are in principle the same as for culturing in other vessels and on particles.

The polymer surface material should not be toxic to the cells to be cultured. We have, for instance recognized, that gas plasma treated polymers built up of acrylo nitrile monomer or acrylate monomer may be toxic, probably due to degradation of the polymer. This may be circumvented by avoiding these kinds of material or by a proper post treatment of the material before it is used.

The invention will now be illustrated by non-limiting experiments. The invention is further defined in the appended claims that are part of the application text.

EXPERIMENTAL PART

Materials

Surfaces (discs): Polycarbonate of bisphenol A and polymethylmethacrylate CD blanks, injection molded at Toolex Alpha AB, Sundbyberg, Sweden. Non-patterned CD blanks, injection molded from Zeonex (a cycloolefin copolymer from Nippon Zeon, Japan) or Luran KR2536 (a styrene-acrylonitrile copolymer (SAN) from BASF, Germany) at Åmic AB, Uppsala,

Sweden). The planar (non-patterned) side of the discs were used in all experiments.

Gases: Oxygen, Argon and synthetic air were from l´Air Liquide, France.

Plasma reactor: Plasma Science, PS0500 (BOC Coating Technology) main adjustable parameters: Radiofrequency (RF) power 0-500 W and gas flow 0-100 or 0-1000 sccm (standard cm^3/min)

Normally the reactor PS0500 is equipped with three electrode plates but after a rebuilding only one plate remained.

Methods

Washing: Before plasma treatment all discs were immersed in pro analysi isopropanol for 2 min, briefly flushed with 99% ethanol and blown dry with house nitrogen. This was done to remove any release agents, antistatic agents etc which might interfere with the plasma treatments.

Plasma treatments: The discs were placed in the plasma reactor in one of two positions; either on a plastic support 20.5 cm from the chamber floor or on glass supports placed on the electrode plate (45 cm from the chamber floor). After evacuation to a base pressure of 60 mTorr, the gas was let in and the gas flow adjusted to the desired level. The RF power was then switched on for the intended time and the reactor chamber was finally vented with ambient air.

Contact angle measurements: Directly after treatment, the equilibrium water-contact angle was measured with the sessile drop method on a Ramé-Hart goniometer bench. For each sample six measurements were made (two sides on each of three droplets). Contact angle measurements were also made after the pieces had been immersed for 2 min in 70% ethanol/water and blown dry with house nitrogen. The measurement was made

within 20 s after the liquid had been applied in order to avoid changes in contact angle e.g. due to evaporation of the droplets.

Check for introduction of cross-links: The polymer material was dissolved in a suitable solvent for original polymer but not for polymer chains cross-linked during the plasma treatment before and after gas plasma treatments. The presence of any insoluble material after treatment was taken as an indication of introduction of cross-links.

Check for introduction of polar oxygen containing groups: Preliminary studies by ESCA showed that the pattern of these groups in the surface changed upon gas plasma treatment in a way suggesting an increase in surface bound oxygen. ESCA can be used to determine the various polar/charged groups that may be of importance for cell culturing.

Storage study: The plasma treated discs were placed in polystyrene Petri dishes and stored under ambient lab conditions. With regular intervals small pieces were cut off and the immediate water-contact angle measured both directly and after immersion in 70% ethanol/water. The cut-off samples were discarded after measurement.

Criteria for acceptance: From the hydrophilicity point of view the preliminary acceptance criterion was that the water-contact angle should be 20° or lower after washing in 70% ethanol.

Cell culture:

Methods for evaluating surfaces:

Cell culture was used as a method for evaluation of the plasma-treated surfaces, since cells pose extremely high demands on their substratum.

Pieces of plasma-treated material were placed in multi-well plates, a suspension of cells in culture medium was added to each well and the plates were incubated in a cell culture atmosphere for various times. Cell adhesion, morphology and proliferation were evaluated microscopically and occasionally by the use of immunocytochemistry against cell proliferation markers and adhesive contacts. Preliminary results have revealed that the plasma-treated surfaces can be used for cell culture and that the cells exhibit necessary characteristics for optimal behaviour. Such characteristics include adhesion of nearly all of the plated cells in a evenly pattern, proper cell spreading on the material surface, signs of normal cell motility and cell division. Pathological signs including vacoles, excessive amounts of lysosomal granulae, blebbing or membrane destruction were insignificant. The cell lines tested so far include MRC5 (normal lung fibroblasts), HeLa (cervix carcinoma cells of epithelial-like origin), Chang (hepatoma, liver cells). The cells have been selected so that they will cover a wide range of demands on the surfaces. Non-anchorage dependent cells put very small demands on the surfaces as such. An example of such cells (Raji lymphoma), have been successfully cultured in the presence of the plasma-treated surfaces.

Our results suggest efficient cell culturing properties for our inventive plasma-hydrophilised surfaces. Efficient cell adhesion and growth could be accomplished for fibroblast-like cells on surfaces having water contact angels in the interval 10-40° and for epithelial like cells in the interval 5-40°.

RESULTS

Planar CD discs plasma-treated

Plasma Science PS0500 reactor with one electrode plate

Samples placed on a polypropylene support 20 cm from the reactor chamber floor in the center of the chamber

Disc material	Gas	Gas flow set value sccm	RF power W	Power/flow W/sccm	Plasma time Min	Contact angle direct	Contact angle washed in 70% EtOH
poly-carbonate	Oxygen	15	500.	33,33333	5	3	25
poly-carbonate	Oxygen	15	300	20	5	3	38
poly-carbonate	Oxygen	10	500	50	5	3	11
polycarbonate	Oxygen	10	300	30	5	4	31
poly-carbonate	Oxygen	5	500	100	5	3	5
polycarbonate	Oxygen	5	300	60	5	4	16
poly-carbonate	Air (synth)	25	500	20	5	4	17
poly-carbonate	Air (synth)	25	300	12	5	10	33
polycarbonate	Air (synth)	5	500	100	5	3	2
poly-carbonate	Air (synth)	5	300	60	5	4	13
Poly-carbonate	Argon	100	500	5	5	25	48
Poly-carbonate	Argon	100	300	3	5	27	56
Poly-carbonate	Argon	25	500	20	5	4	18
Poly-carbonate	Argon	25	300	12	5	9	39
Poly-carbonate	Argon	5	500	100	5	4	3
Poly-carbonate	Argon	5	300	60	5	4	9
Zeonex	Oxygen	100	500	5	5	20	29
Zeonex	Oxygen	100	300	3	5	17	34

Zeonex	Oxygen	50	500	10	5	10	5
Zeonex	Oxygen	50	300	6	5	15	28
Zeonex	Oxygen	25	500	20	5	7	4
Zeonex	Oxygen	25	300	12	5	11	10
Zeonex	Oxygen	5	500	100	5	4	2
Zeonex	Oxygen	5	300	60	5	4	4
Zeonex	Air (synth)	100	500	5	5	16	29
Zeonex	Air (synth)	100	300	3	5	16	36
Zeonex	Air (synth)	50	500	10	5	9	6
Zeonex	Air (synth)	50	300	6	5	15	26
Zeonex	Air (synth)	25	500	20	5	5	4
Zeonex	Air (synth)	25	300	12	5	9	6
Zeonex	Air (synth)	5	500	100	5	8	4
Zeonex	Air (synth)	5	300	60	5	5	4
SAN	Oxygen	100	500	5	5	8	27
SAN	Oxygen	100	300	3	5	11	23
SAN	Oxygen	50	500	10	5	8	7
SAN	Oxygen	50	300	6	5	9	22
SAN	Oxygen	25	500	20	5	5	7
SAN	Oxygen	25	300	12	5	7	14
SAN	Oxygen	5	500	100	5	4	2
SAN	Oxygen	5	300	60	5	5	3
SAN	Air (synth)	100	500	5	5	8	27
SAN	Air (synth)	100	300	3	5	8	26
SAN	Air (synth)	50	500	10	5	7	8
SAN	Air (synth)	50	300	6	5	8	26
SAN	Air (synth)	25	500	20	5	4	5
SAN	Air (synth)	25	300	12	5	6	12
SAN	Air (synth)	5	500	100	5	5	4
SAN	Air (synth)	5	300	60	5	4	4
PMMA	Air (synth)	50	500	10	5	20	40
PMMA	Air (synth)	50	300	6	5	39	53
PMMA	Air (synth)	25	500	20	5	8	21
PMMA	Air (synth)	25	300	12	5	26	44
PMMA	Air (synth)	10	500	50	5	4	4
PMMA	Air (synth)	10	300	30	5	6	14
PMMA	Air (synth)	5	500	100	5	8	4

PMMA	Air (synth)	5	300	60	5	5	3
PMMA	Oxygen	50	500	10	5	29	54
PMMA	Oxygen	50	300	6	5	39	52
PMMA	Oxygen	25	500	20	5	11	40
PMMA	Oxygen	25	300	12	5	31	53
PMMA	Oxygen	10	500	50	5	5	10
PMMA	Oxygen	10	300	30	5	7	45
PMMA	Oxygen	5	500	100	5	4	4
PMMA	Oxygen	5	300	60	5	4	7

C L A I M S

1. A method for rendering a surface covered by a polymer material (plastics) more hydrophilic by treatment in a gas plasma of a non-polymerizable gas, **characterized** in that the intensity of the plasma is selected so that the surface becomes permanently more hydrophilic, and with the optional step that the surface subsequently is washed with a solvent selected from water, a water-miscible solvent and mixtures thereof.

2. The method according to claim 1, **characterized** in that the plasma intensity is ≥ 5 W/cm^3/min, in particular with a power ≥ 250 W and a flow ≤ 50 cm^3/min.

3. The method of any one of claims 1-2, **characterized** in that
   (a) the polymer material has been selected among plastics having an immediate water-contact angle $\geq 20°$ and
   (b) the plasma treatment conditions are set so that the immediate water-contact angle after plasma treatment and a subsequent wash with ethanol (70 % w/w) becomes $\leq$ 30°, such as $\leq 20°$.

4. The method of any one of claims 1-3, **characterized** in that the polymer material on the surface is selected among
   (a) polymers/copolymers in which the monomers are unsaturated such as (i) alkenes/alkadienes (such as ethen, propen, butadiene and including substituted forms such as vinyl ethers, polyfluorovinyl hydrocarbons (for instance tetrafluoroethylene), and acids, esters, amides, nitriles etc containing one or more alkene groups, for instance various methacryl/acryl compounds; (ii) vinyl aryl compounds in which the vinyl group is bound to aryl (such as mono-, di- and trivinyl benzenes) that optionally may be substituted with for instance lower alkyl groups (C1-6); etc;
   (b) condensation polymers/copolymers in which the monomomers are selected from compounds exhibiting two

or more groups selected among amino, hydroxy, carboxy etc groups.

5. The method of any one of claims 1-4, **characterized** in that the plasma is induced by radio- and/or microwaves.

6. The method of any one of claims 1-5, **characterized** in that the plasma gas is oxygen, nitrogen, or a noble gas, such as argon, or a mixture of these gases.

7. The method of any one of claims 1-6, **characterized** in that subsequent to the plasma treatment the surface is derivatized to exhibit positively, negatively and amphoteric groups; hydroxy groups, bioaffinity groups, chelating groups etc.

8. The method of any one of claims 1-7, **characterized** in that at least part of the surface is fabricated into a liquid transportation system of a microfabricated device.

9. A naked plasma treated surface of plastics **characterized** in that the surface has an immediate water-contact angle ≤ 30°, such as ≤ 20°, said water-contact angle being changed less than ± 20% and/or less than ± 5° upon washing with ethanol/water mixture (70% w/w).

10. The naked surface of claim 9, **characterized** in that the surface is made up of a polymer material selected from
(a) polymers/copolymers in which the monomers are unsaturated such as (i) alkenes/alkadienes (such as ethen, propen, butadiene and including substituted forms such as vinyl ethers, polyfluorovinyl hydrocarbons (for instance tetrafluoroethylene), and acids, esters, amides, nitriles etc containing one or more alkene groups, for instance various methacryl/acryl compounds; (ii) vinyl aryl compounds (such as mono-, di- and trivinyl benzenes) that optionally may be substituted with for instance lower alkyl groups (C1-6); etc;

(b) condensation polymers/copolymers in which the monomomers are selected from compounds exhibiting two or more groups selected among amino, hydroxy, carboxy etc groups;

said polymer material optionally being cross-linked and/or optionally being a mixture of two or more kinds of polymers/copolymers.

11. The naked surface of any one of claims 9-10, **characterized** in that the polymer material before having been gas plasma treated exhibits an immediate water-contact angel ≥ 30°.

12. The naked surface of any one of claims 9-11, **characterized** in that at least part of the naked surface is part of a liquid transportation system of a microfabricated device.

13. The use of the surface defined in any one of claims 9-11 and/or obtained by the method defined in any one of claims 1-8 for culturing cells.

14. A kit comprising

(a) a microfabricated device comprising a surface (i) which is manufactured from a synthetic polymer material (plastics) and (ii) on which there are at least one chamber and/or at least one channel, and

(b) a fluorescent substance to be detected in the device, **characterized** in that the plastics has a fluorescence that is non-significant with respect to the fluorescence, in particular with the fluorescence intensity of the plastics being < 50 % of the fluorescent intensity of the substance at the wavelength at which the substance fluoresces.

15. A method for culturing cells that in at least some part of their life cycle require attachment to a substrate surface, **characterized** in that the substrate surface

provides a plastic surface having an immediate water-contact angle ≤ 30°.

16. The method of claim 15, **characterized** in that the plastic surface having an immediate water-contact angle ≤ 30° has been obtained by the gas plasma treatment method defined in claims 1-8 of a surface made of plastics.

17. The method of any one of claims 15-16, with the proviso that for anchorage-dependent cells the conditions have been selected so that at least 30 % of the plated cells adhere to the substrate surface.

18. The method of any one of claims 15-17, **characterized** in that at most 15 % of serum is present in the culture medium.

19. The method of any one of claims 15-18, **characterized** in that culturing is taking place during a time period permitting the number of cells to be at least duplicated.

20. The method of any one of claims 15-19, **characterized** in that the cell culturing is carried out in a chamber of a microfabricated device.

21. A microfabricated device in which the liquid transportation system is formed in/on a polymer material (plastics) that comprises a polymerised aliphatic monomer containing unsaturation.

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

ROLLINS, Anthony, John
Nycomed Amersham PLC
Amersham Laboratories
White Lion Road
Amersham, Buckinghamshire HP7 9LL
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year)
20 November 2000 (20.11.00)

Applicant's or agent's file reference
PU9909

IMPORTANT NOTIFICATION

International application No.
PCT/EP00/02632

International filing date (day/month/year)
24 March 2000 (24.03.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
GYROS AB
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3. Further observations, if necessary:
Please note that the applicant in Box I has assigned all his rights to the applicant indicated in Box II.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
F. Baechler

Telephone No.: (41-22) 338.83.38

U.S -

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PU9909-PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 02632	24/03/2000	24/03/1999
Applicant		
AMERSHAM PHARMACIA BIOTECH AB e		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. —

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/02632

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8

   Making a plastics surface permanently more hydrophilic by the selected intensity of a gas plasma treatment

   1.1. Claims: 9-13
   A plasma treated hydrophilic surface whose water-contact angle changes less than 20% upon washing

   1.2. Claims: 15-20
   A hydrophilic plastics surface used for culturing cells by their attachment

   1.3. Claim : 21
   A microfabricated liquid transportation system

2. Claim : 14

   A microfabricated device with a plastics surface having a fluorescence intensity less than 50% of the fluorescence intensity of a substance detected in the device

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/02632

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B29C59/14 C12N5/00 B01L3/00 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B29C C12N B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	DE 37 12 491 A (APPLIED MEMBRANE TECH) 15 October 1987 (1987-10-15) page 4, line 67 -page 5, line 26 page 7, line 40 -page 8, line 3 page 8, line 40 -page 9, line 2 page 9, line 12 - line 60; claims; figures ---	1-8, 15-17,21 9-13
X	EP 0 467 639 A (ITOH C FINE CHEMICAL CO ;EC CHEM IND CO (JP); KOGOMA MASUHIRO (JP)) 22 January 1992 (1992-01-22) page 4, line 53 -page 5, line 5	1,2,4-6
Y	page 5, line 10 - line 27; claims; figures ---	9-13
X	US 4 741 619 A (HUMPHRIES GILLIAN M K ET AL) 3 May 1988 (1988-05-03) the whole document ---	1,3-12
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2001	22. 01. 01
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Labeeuw, R

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/02632

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 111 795 A (NIKKA CHEMICAL IND CO LTD ;EMORI & CO LTD (JP); SHINETSU CHEMICAL) 27 June 1984 (1984-06-27) page 4, line 27 -page 5, line 6 page 6, line 13 - line 21 page 8, line 16 -page 9, line 10 ---	1,2,5-7
X	WO 97 21090 A (GAMERA BIOSCIENCE) 12 June 1997 (1997-06-12)	15-21
A	page 19, line 8 -page 20, line 10; figures ---	1-13
P,X	WO 99 58245 A (AMERSHAM PHARM BIOTECH AB ;ALLMER KLAS (SE); ANDERSSON PER (SE); L) 18 November 1999 (1999-11-18) cited in the application page 2, line 14 - line 21; claims 1-4,11,12; figures; example 1 ---	1-4, 8-13, 15-17, 20,21
A	GB 2 061 969 A (SHINETSU CHEMICAL CO) 20 May 1981 (1981-05-20) cited in the application the whole document ---	1,9
X	EP 0 106 662 A (DYNATECH LAB) 25 April 1984 (1984-04-25) the whole document ---	14
X	DE 197 39 119 A (UNIV SCHILLER JENA) 11 March 1999 (1999-03-11) column 2, line 1 - line 18; claim 1; figures ---	14
P,X	US 6 027 695 A (OLDENBURG KEVIN R ET AL) 22 February 2000 (2000-02-22) the whole document and in particular column 1 line 36 - line 61, column 3 line 4 - line 23 and column 7 line 66 - column 8 line 29 ---	14
A	US 5 399 316 A (YAMADA TAKASHI) 21 March 1995 (1995-03-21) column 4, line 60 -column 5, line 12 column 5, line 43 - line 53; figure -----	14

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/02632

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3712491 A	15-10-1987	AU 7135087 A	15-10-1987
		DK 185787 A	12-10-1987
		JP 62294407 A	21-12-1987
EP 0467639 A	22-01-1992	JP 3063769 B	12-07-2000
		JP 4074525 A	09-03-1992
		CA 2047147 A	18-01-1992
		DE 69128502 D	05-02-1998
		DE 69128502 T	23-07-1998
		KR 169490 B	20-03-1999
		US 5124173 A	23-06-1992
US 4741619 A	03-05-1988	EP 0290125 A	09-11-1988
EP 0111795 A	27-06-1984	JP 59106567 A	20-06-1984
		JP 59106568 A	20-06-1984
		DE 3377148 D	28-07-1988
WO 9721090 A	12-06-1997	AU 702403 B	18-02-1999
		AU 1283397 A	27-06-1997
		CA 2239613 A	12-06-1997
		CN 1208464 A	17-02-1999
		EP 0865606 A	23-09-1998
		NO 982563 A	05-08-1998
		AU 4144897 A	06-03-1998
		EP 0917648 A	26-05-1999
		WO 9807019 A	19-02-1998
		US 6143248 A	07-11-2000
WO 9958245 A	18-11-1999	AU 3624399 A	29-11-1999
		GB 2350678 A	06-12-2000
		GB 2341924 A	29-03-2000
GB 2061969 A	20-05-1981	JP 1338119 C	29-09-1986
		JP 56061434 A	26-05-1981
		JP 60039290 B	05-09-1985
		DE 3039852 A	17-09-1981
		FR 2467865 A	30-04-1981
		NL 8005814 A	27-04-1981
EP 0106662 A	25-04-1984	AU 564381 B	13-08-1987
		AU 1929583 A	19-04-1984
		DE 3378545 D	29-12-1988
		ES 525541 D	16-12-1984
		ES 8501998 A	16-03-1985
		ES 535979 D	01-03-1987
		ES 8703753 A	16-05-1987
		JP 2078438 C	09-08-1996
		JP 3040818 B	20-06-1991
		JP 59132335 A	30-07-1984
		US 4725388 A	16-02-1988
DE 19739119 A	11-03-1999	NONE	
US 6027695 A	22-02-2000	NONE	
US 5399316 A	21-03-1995	JP 5253498 A	05-10-1993
		DE 4307735 A	23-09-1993

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Nakan, Bergander
GYROS AB
Uppsala Science Park
75183 Uppsala
SUEDE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	27.04.2001
REPLY DUE	**within 1 month(s) and 7 days** from the above date of mailing

Applicant's or agent's file reference	International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PU9909-PCT	PCT/EP00/02632	24/03/2000	24/03/1999

International Patent Classification (IPC) or both national classification and IPC

C08J7/00

Applicant

GYROS AB

1. This written opinion is the **second** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 24/07/2001.

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner

Kujat, C

Formalities officer (incl. extension of time limits)

Novoa, C
Telephone No. +49 89 2399 2718

I. Basis of the opinion

1. With regard to the **elements** of the international application (Replacement *sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, pages:

1-25 as originally filed

Claims, No.:

1-21 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-8,14-16,20,21
Inventive step (IS)	Claims	9-13,17-19
Industrial applicability (IA)	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item VIII

Certain observations on the international application

1.1 **Independent claim 1** lacks clarity (Art. 6 PCT) for the following reasons:

1.1.1 The feature "a surface" is not clear because by definition a surface is two-dimensional. However, a physical entity, which only can be "covered by a polymer", by definition is three-dimensional.
It would be clear to use the term "substrate" (dependent claim 15) instead of "surface".
This objection also applies to dependent **claims 4, 7 to 12, independent claim 13 and dependent claim 16.**

1.1.2 The feature "(plastics)" does not meet the requirements of article 6 PCT because it is placed in brackets. Therefore, it is unclear whether this feature shall be limiting or not. This objection also applies to independent **claims 14 and 21.**

1.1.3 The negative feature "non-polymerizable gas" does not meet the requirements of article 6 PCT because the extent of protection is not clear. A positive definition, e.g. an enumeration of gases which are of the non-polymerizable kind, such as given in claim 6, would be clear.

1.1.4 The feature "the intensity of the plasma is selected so that ... more hydrophilic" does not meet the requirements of article 6 PCT because it attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It appears possible to define the invention in more concrete terms, see e.g. the values given in dependent claim 2.

1.2 The additional feature of dependent **claim 2**, "conditions are set so that ..." does not meet the requirements of article 6 PCT because it is not clear which conditions, e.g. plasma intensity, frequency, power or flow of gas supply, are referred to.
Moreover, the feature "conditions are set so that ..." also attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It

appears possible to define the invention in more concrete terms, see e.g. the values given in dependent claim 2.

1.3 The additional features of dependent **claim 7** do not meet the requirements of article 6 PCT because it is not clear by means of which method steps the surface is derivatized.
Moreover, the feature "positively, negatively groups" is not clear: Apart from the grammatical inconsistency, in case that "positive, negative groups" are meant, it is not clear to exactly which kind of (chemical?) groups this feature shall refer. It would be clear to list examples.
Furthermore, the feature "bioaffinity groups" lacks clarity, as it is not properly defined, e.g. by means of examples.

1.4 The additional feature "micro fabricated device" in independent **claims 14 and 21** and dependent **claims 8, 12 and 20** does not meet the requirements of article 6 PCT because it is not clear in how far a micro fabricated device differs from a conventionally fabricated device.
It would be clear to add the definition given on page 12, lines 3 to 6, to said claims.

1.5 The feature "naked... surface" in **independent claim 9** does not meet the requirements of article 6 PCT because it is not clear by means of which criterion a surface must be considered naked or not-naked, respectively. Moreover, the **scope of protection** of claim 9 is not clear at all, because the feature "a surface" by definition is two-dimensional. However, a physical entity, to which such a product claim can only refer, by definition is three-dimensional.

1.6 The feature of **independent claim 13**, "culturing cells" does not meet the requirements of article 6 PCT because it is not clear which scope of protection the term "culturing" shall define.

1.7 Independent product **claim 14** does not meet the requirements of Art. 6 PCT, since the wording of the characterizing part does not make sense: "the plastics has a fluorescence that is non-significant with respect to the fluorescence" is contradictory. The plastics on the one hand is supposed to have a feature

(=fluorescence), which on the other hand it is not supposed to have, since that feature shall be "non-significant".
In addition to that, the negative feature "non-significant" itself is unclear. See "PCT-Gazette", Section IV, III-4.12, according to which the subject-matter of a claim must be defined by positive features.

1.8 The feature of **independent claim 15**, "that in at least some part of their life cycle require attachment to a substrate surface" does not meet the requirements of article 6 PCT because it is not clear which kind of cells are claimed.

1.9 The additional feature of dependent **claim 17**, "conditions have been selected ..." does not meet the requirements of article 6 PCT because it is not clear which conditions, e.g. plasma intensity, frequency, power or flow of gas supply, are referred to.
Moreover, the feature "conditions have been selected ..." also attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It appears possible to define the invention in more concrete terms, by specifying the values which define these "conditions", see e.g. the values given in dependent claim 2.

1.10 The additional feature of dependent **claim 18**, "serum" and "culture medium" are not clear because no definition is given.

Re Item V
Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Not-withstanding the clarity objections raised under Item VIII, novelty and inventive step will now be assessed. Reference is made to the following documents:

D1: DE 3712491
D2: WO 97/21090
D3: EP 106662

Independent Claim 1:

2.1 Document **D1**, which is considered the most relevant prior art for **claim 1**, discloses:
A method (page 2, line 20) for rendering a surface (page 2, line 3) covered by (The substrate is already made of polymer material, see page 2, line 13. Therefore, the surface of this substrate is considered being covered by a polymer material.) a polymer material (page 2, line 13) more hydrophilic (page 2, line 3) by treatment in a gas plasma (page 2, line 7) of a non-polymerizable gas (page 2, line 7), where the intensity of the plasma is selected (page 4, line 64 "Steuerung bzw. Regelung", page 5, lines 24 and 25 "Bedingungen..., die zum Ändern der Oberflächencharakteristik von hydrophob nach ... dauerhaft hydrophil ausreichen", page 6, line 47 "... so geregelt, daß die höchste Energiedichte ... aufrechterhalten bleibt", page 9, lines 20 to 27 "Abgabeleistung 60 W, Gasstrom Luft 8,5 sccm") so that the surface becomes permanently (page 2, line 1) more hydrophilic (page 2, line 1).
Therefore, the subject-matter of independent claim 1 is not new versus D1 (Art. 33(2) PCT).

2.2 Further, the subject-matter of independent claim 1 lacks novelty versus the disclosures of any of US 4741619, EP 476639 and EP 111795. These documents have been cited in the International Search Report and reference is made to the parts of these documents mentioned in the International Search Report.

2.3 Dependent claims 2 to 8 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty, the reasons being as follows:

2.3.1 Concerning the additional features of dependent **claim 2**, D1 already discloses a plasma intensity of greater than 5 W/cm^3/min (page 9, lines 20 to 27: 60 W divided by 8.5 sccm equals 7.0588 W/cm^3/min).

2.3.2 The additional features of dependent **claim 3** have already been disclosed in D1 (page 2, line 13 "polyolefin", which includes both polypropylene and polyethylene. Both materials have an immediate water-contact angle of

greater than 90°, see description, page 8. The immediate water-contact angle after treatment is down to 21°, page 8, line 28).

2.3.3 The additional features of dependent **claim 4** have already been disclosed in D1 (page 2, line 14).

2.3.4 The additional features of dependent **claim 5** have already been disclosed in D1 (page 9, lines 18 and 67).

2.3.5 The additional features of dependent **claim 6** have already been disclosed in D1 (page 2, lines 47 and 48).

2.3.6 The additional features of dependent **claim 7** have already been disclosed in D1 (page 8, line 66).

2.3.7 The additional features of dependent **claim 8** have already been disclosed in D1: page 2, lines 3 and 4, and page 3, line 8, disclose a micro-fabricated device, because the device "Mikrofiltrations-Membran" or "Zellkultur-Einheit" is described as being "mikroporös". Moreover, that membrane forms part of a liquid transportation system (see page 3, line 24: "Permeat..., welches durch diese Hohlfaser dringt").

Independent Claim 9:

3.1 The subject-matter of independent claim 9 differs from the disclosure of document D1 in that the water-contact angle (is) changed less than ± 20% upon washing with ethanol/water mixture.

3.2 However, such a characteristic seems to be implicitly disclosed in D1, because D1 highlights the low contact angle (page 9, lines 30 and 31: "Benetzbarkeiten ... haben sich bemerkenswert verbessert") upon washing with wafer-washing-solution (page 9, line 30: "Wafer Wasch/Kühlmittel ... Aqua Kool 7X").
Both the ethanol/water mixture and the wafer-washing solution of D1 are considered solvents.

3.3 Therefore, the disclosure of an equivalent substance in claim 9 is not considered involving an inventive step (Art. 33(3) PCT).

3.4 Dependent claims 10 to 12 do not appear to contain any additional features, which in combination with the features of any claim to which they refer meet the requirements of the PCT with regard to inventive step, the reasons being as follows, and the problem-solution-approach-analysis remaining the same, the distinguishing features over D1 remaining identical:

3.4.1 The additional features of dependent **claim 10** have already been disclosed in D1 (page 2, line 14).

3.4.2 The additional features of dependent **claim 11** have already been disclosed in D1 (page 2, line 13 "polyolefin", which includes both polypropylene and polyethylene. Both materials have an immediate water-contact angle of greater than 90°, see description, page 8.

3.4.3 The additional features of dependent **claim 12** have already been disclosed in D1: page 2, lines 3 and 4, and page 3, line 8, disclose a micro-fabricated device, because the device "Mikrofiltrations-Membran" or "Zellkultur-Einheit" is described as being "mikroporös". Moreover, that membrane forms part of a liquid transportation system (see page 3, line 24: "Permeat..., welches durch diese Hohlfaser dringt").

Independent Claim 13:

4. Independent claim 13, which relates to the use of the product obtained by any of claims 9 to 11, can not be considered involving an inventive step (Art. 33(3)) for the same reasons as explained in paragraph 3.

Independent Claim 14:

5.1 Concerning independent claim 14, document **D3** discloses a kit (page 1, line 1:

"laboratory equipment") comprising a micro fabricated device (page 1, line 23: Since it the device disclosed in D3 is a micro test plate, it is considered being micro-fabricated.) comprising a surface which is manufactured from a synthetic polymer material (page 2, line 14) and on which there is at least one chamber (page 2, line 2: "well") and a fluorescent substance (page 1, line 10: "fluorescently labeled reactant") to be detected in the device wherein the plastics has a fluorescence that is non-significant (page 14, line 28: "no detectable light emissions") with respect to the fluorescence *of the fluorescent substance*. Therefore, the subject-matter of independent claim 14 is not new versus D3 (Art. 33(2) PCT).

5.2 Moreover, **D3** also discloses that the fluorescence intensity of the plastics is <50% (page 14, line 28: "no detectable light emissions") of the fluorescent intensity of the substance at the wavelength at which the substance fluoresces.

5.3 X-document DE 19739119 cited in the Search Report is also novelty-destroying for the subject-matter of independent claim 14. See the passages mentioned in the Search Report.

Independent Claim 15:

6.1 Document **D2**, which is considered representing the most relevant prior art for independent claim 15, discloses:
A method for culturing cells that in at least some part of their life cycle require attachment to a substrate surface (page 52, lines 17 to 22 and page 54, lines 1 to 3) where the substrate surface provides a plastic surface (page 5, lines 30 and page 19) having an intermediate water contact angle ≤ 30° (All the materials disclosed on page 19 fulfill this requirement. See description, page 8).
Therefore, the subject-matter of independent claim 15 is not new versus D2 (Art. 33(2) PCT).

6.2 Dependent claims 16 and 20 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty, the reasons being as follows:

6.2.1 The additional features of dependent **claim 16** have already been disclosed in D2 (page 20, lines 8 to 10, line 14 and lines 31 and 32).

6.2.2 The additional features of dependent **claim 20** have already been disclosed in D2 (page 5, lines 24 and 28).

6.3 Dependent claims 17 to 19 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:

6.3.1 Concerning dependent **claim 17**, D2 already discloses that for anchorage dependent cells the conditions have been selected so that the cells adhere to the substrate surface (page 20, lines 5 and 6, page 54, lines 5 to 12 and page 55, line 6). It is therefore considered an obvious variation falling within the scope of the man skilled in the art to make sure that at least 30% of these cells adhere.

6.3.2 The additional features of dependent **claims 18 and 19** relate to well known practices in the art of culturing cells and are therefore considered obvious for the person skilled in the art.

Independent Claim 21:

7.1 Document D2, which is considered representing the closest prior art for the subject-matter of independent claim 21, discloses
a micro fabricated device (page 5, lines 24 and 28) in which the liquid transportation system (page 23, lines 21 and 22) is formed in a polymer material ((page 5, lines 30 and page 19)) that comprises a polymerised aliphatic monomer containing unsaturation (The plastic materials disclosed in D2 fall within the definition given on pages 8 and 9 of the description.).

7.2 Therefore, the subject-matter of independent claim 21 lacks novelty versus the disclosure of D2.

Re Item VII

Certain defects in the international application

8. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents **D1, D2 and D3** is not mentioned in the description, nor are these documents identified therein.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Bergander, Hakan
GYROS AB
Uppsala Science Park
S-75183 Uppsala
SUEDE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 04.07.2001
Applicant's or agent's file reference PU9909-PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02632	24/03/2000	24/03/1999

Applicant
GYROS AB

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Vatel, M

Tel.+49 89 2399-8225

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PU9909-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02632	24/03/2000	24/03/1999
International Patent Classification (IPC) or national classification and IPC C08J7/00		
Applicant GYROS AB		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/10/2000	04.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Kujat, C Telephone No. +49 89 2399 2360

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/02632

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1,4-25	as originally filed			
2,3,3a	as received on	16/06/2001	with letter of	13/06/2001

Claims, No.:

1-22	as received on	16/06/2001	with letter of	13/06/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,3,8,10-14,19-21
	No:	Claims	1,4,-7,9,15-18,22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	2,3,8,10-14,19-21
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item VIII

Certain observations on the international application

1.1 **Independent claim 1** lacks clarity (Art. 6 PCT) for the following reasons:

1.1.1 The feature "said surface containing a liquid transportation system in which there is a channel with a depth ≤ 1000 µm" in the method claim 1 relates to characteristics of a substrate surface rather than clearly defining the method in terms of its method steps. The intended limitations and the category of the claim are therefore not clear from this claim, contrary to the requirements of Article 6 PCT.

Since claim 1 appears to be directed to the application of a "method for rendering..." to a specific product ("a surface containing a liquid transportation system..."), claim 1 will be construed during the subsequent assessment of novelty and inventive step having the meaning:

A method for rendering a substrate surface made in plastic material more hydrophilic, **said surface containing a liquid transportation system in which there is a channel with a depth ≤ 1000 µm**, by treatment in a gas plasma... .

1.1.2 The feature "the intensity of the plasma is selected so that ... more hydrophilic" does not meet the requirements of article 6 PCT because it attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It is possible to define the invention in more concrete terms, see e.g. the values given in dependent claims 2 and 3.

1.1.3 Moreover, the optional feature "the surface subsequently is washed with a pure solvent selected from water, a water-miscible solvent and mixtures thereof" does not meet the requirements of article 6 PCT because it contains a contradiction: A mixture of water and a water-miscible solvent cannot be a "pure" solvent.

1.1.4 Further, the feature "permanently more hydrophilic" does not meet the requirements of article 6 PCT because it is not clear by which means "permanently more hydrophilic" differs from "more hydrophilic". A test

procedure defining criteria which enable the skilled man to distinguish a "permanently more hydrophilic" surface from a "more hydrophilic" surface would meet the criteria of Art. 6 PCT.

1.2 The additional feature of dependent **claim 4**, "conditions are set so that ..." does not meet the requirements of article 6 PCT because it is not clear which conditions, e.g. plasma intensity, frequency, power or flow of gas supply, are referred to.
Moreover, the feature "conditions are set so that ..." also attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It appears possible to define the invention in more concrete terms, see e.g. the values given in dependent claim 2. This feature is unclear as it appears to be the result of plasma treatment conditions which are not further defined.

1.3 The feature of **independent claim 14**, "the culturing of cells" does not meet the requirements of article 6 PCT because it is not clear which scope of protection the term "culturing" shall define. A proper definition, e.g. as given on page 16 of the description would render claim 14 clear.

1.4 The feature "in uncoated form" in **independent claim 10** does not meet the requirements of Art. 6 PCT, since it is not clear whether "uncoated" refers to the state of the surface before or after plasma treatment. Since the plasma treatment in the application in suit is performed with a non-polymerizable gas (see independent claim 1), no coating will be applied during the plasma treatment. "Uncoated" is therefore construed as having the meaning "immediately after plasma treatment".

1.5 The feature of **dependent claim 19**, "the substrate surface enables at least 30% of the plated cells to adhere" does not meet the requirements of article 6 PCT because it attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It appears possible to define the invention in more concrete terms, e.g. in terms of how the surface has been obtained or which structure/texture it has.

1.6 The additional feature "micro fabricated device" in dependent **claim 22** does not

meet the requirements of article 6 PCT because it is not clear in how far a micro fabricated device differs from a conventionally fabricated device.
It would be clear to add the definition given on page 12, lines 3 to 6, to said claims.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Not-withstanding the clarity objections raised under Item VIII, novelty and inventive step will now be assessed. Reference is made to the following documents:

D1: WO 97/21090
D2: DE 3712491
D3: EP 467639

Independent Claim 1:

2.1 Document **D1** discloses a substrate surface (pages 17 and 19: injection moulded plastic disc) which contains a liquid transportation system (page 1, line 17: "utilizing the centripetal forces resulting from rotation of the platform to motivate fluid movement") in which there is a channel (page 1, line 18: "fluid movement through micro channels") with a depth ≤ 1000 µm (page 12, line 6: "is less than 500 µm").
D1 further discloses that there is a strong correlation between water contact angle and cell adsorption, with hydrophilic surfaces showing significantly less cell adsorption than hydrophobic surfaces (page 20, lines 9 and 10) and that it is desirable to increase the hydrophilicity of such a surface (page 20, line 14). For doing so, D1 suggests the use of inert gas plasmas (page 20, line 32).

Due to the disclosure of such a substrate in D1, a "method for rendering a substrate surface made in plastic material more hydrophilic by treatment in a gas plasma of a non-polymerizable gas" is also disclosed by D1.

Further, the skilled person is well aware that he must precisely select certain parameters of the plasma (e.g. intensity, power, frequency, gas flow-rate) during plasma treatment in order to reproducibly obtain a desired result.

2.2 Therefore, the subject-matter of independent **claim 1 is not new versus D1** (Art. 33(2) PCT).

2.3 Dependent **claims 2, 3 to 8** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of **inventive step**, the reasons being as follows:

2.3.1 The additional features of dependent **claims 2 and 3** appear to relate to common parameters in plasma treatment which appear to be obvious for the skilled person. See e.g. **D2** (plasma intensity of greater than 5 W/cm^3/min (page 9, lines 20 to 27: 60 W divided by 8.5 sccm equals 7.0588 W/cm^3/min)) or **D3** discloses a power ≥ 250 W (page 5, line 3).

2.3.2 Concerning the additional features of dependent **claim 8,** the derivatization of the surface appears to be a common practice in the field of plasma treatment, which is therefore devoid of inventive activity.

2.4 Dependent **claims 4, 5, 9** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of **novelty**, the reasons being as follows:

2.4.1 Apart from the disclosure of materials in **D2** (page 2, line 13 "polyolefin", which includes both polypropylene and polyethylene. Both materials have an immediate water-contact angle of greater than 90°, see description, page 8. The immediate water-contact angle after treatment is down to 21°, page 8, line 28), the materials of dependent **claims 4 and 5** have also been disclosed in **D1** (page 17, last paragraph). The feature "(a) the plasma treatment conditions are set so that..." is not discussed as it is not clear (see paragraph 1.2).

2.4.2 The additional features of dependent **claims 6 and 7** have been implicitly

disclosed in **D1** (page 20, line 32): The skilled person in the field of plasma treatment knows well that an inert-gas (or reactive-gas) plasma is induced by a frequency corresponding to radio- or microwaves. He further knows well that the plasma gas is nitrogen or a noble gas for inert-gas plasmas.

2.4.3 The additional feature of dependent **claim 9** has already been disclosed in document **D1** (page 12, line 6: "is less than 500 µm").

Independent Claim 10:

3.1 Document **D2** discloses a substrate surface (surface of the membrane "Mikrofiltrations-Membran", see claim 1) which is made of a plastic material (see claims 4 and 5), which has been plasma treated (see claim 1: "Behandlung mit einem nicht-polymerisierbaren Plasmagas"), where the surface in uncoated form (i.e. directly after plasma-treatment) has an immediate water contact angle ≤ 30° (page 8, line 28).

3.2 The subject-matter of independent claim 10 differs from the disclosure of document **D2** in that the water-contact angle (is) changed less than ± 20% upon washing with ethanol/water mixture (70% w/w). (The feature "a pure ethanol/water mixture" lacks clarity for the reasons given in paragraph 1.1.3).

3.3 This feature per se is unclear for the reasons given in paragraph 1.2 as it appears to be the result of plasma treatment conditions which are not further defined. Not withstanding this objection, the inventive step will now be discussed:
Such a characteristic seems to be implicitly disclosed in D2, because D2 highlights the low contact angle (page 9, lines 30 and 31: "Benetzbarkeiten ... haben sich bemerkenswert verbessert") for wafer-washing-solution (page 9, line 30: "Wafer Wasch/Kühlmittel ... Aqua Kool 7X").
Both the ethanol/water mixture and the wafer-washing solution of D2 are considered solvents.
Moreover, the feature "washing" does not seem to have any limiting effect further than the meaning "bringing into contact with". Since the membrane of **D2** is immersed in a liquid (see e.g. claims 27 and 28) during its intended use, it is assumed that "washing" takes place during that use. The information relating to

the wettability ("Benetzbarkeit") on page 9, line 30 must therefore be interpreted in the light of this intended use.

3.4 Therefore, the disclosure of an equivalent substance in **claim 10 is not considered involving an inventive step** (Art. 33(3) PCT).

3.5 Dependent **claims 11 to 13** do not appear to contain any additional features, which in combination with the features of any claim to which they refer meet the requirements of the PCT with regard to inventive step, the reasons being as follows, and the problem-solution-approach-analysis remaining the same, the distinguishing features over D1 remaining identical:

3.5.1 The additional features of dependent **claim 11** have already been disclosed in D2 (page 2, line 14).

3.5.2 The additional features of dependent **claim 12** have already been disclosed in D2 (page 2, line 13 "polyolefin", which includes both polypropylene and polyethylene. Both materials have an immediate water-contact angle of greater than 90°, see description, page 8.

3.5.3 The additional features of dependent **claim 13** render independent claim 10 unclear. It is not possible to say whether the substrate surface disclosed in **D1** meets the requirement relating to the immediate water contact angle defined in claim 10 (thus, destroying novelty of claim 13).

Independent Claim 14:

4. Independent claim 14, which relates to the use of the product obtained by any of claims 1 to 13, can not be considered involving an inventive step (Art. 33(3)) for the lack of clarity explained in paragraph 1.3.

Independent Claim 15:

5.1 Concerning independent claim 15, document **D1** discloses a kit comprising a micro fabricated device (page 5, line 24: "micro platform disk") comprising a substrate surface which is made in a plastic material (page 17, lines 30 to 34) and which comprises a liquid transportation system (page 1, line 17: "utilizing the centripetal forces resulting from rotation of the platform to motivate fluid movement") comprising a channel (page 1, line 18: "fluid movement through micro channels") which has a depth ≤ 1000 µm (page 12, line 6: "is less than 500 µm") and a fluorescent substance * to be detected in the device.

* Since the device disclosed in **D1** contains fluorescent detecting means (page 11, line 5) and since D1 relates to fluorescence excitation (page 31, line 2), a fluorescent substance is used as a fluid in a corresponding application of the micro fabricated device of D1.

5.2 The additional feature "the fluorescence intensity of the plastic material being < 50% of the fluorescent intensity of the substance at the wavelength at which the substance fluoresces is considered an implicit feature of the micro fabricated device of D1, since it would not work otherwise.

5.3 Therefore, the subject-matter of independent **claim 15 is not new** versus **D1** (Art. 33(2) PCT).

5.4 The additional features of dependent **claim 16** have already been disclosed in **D1** (see page 17, lines 30 to 34).

Independent Claim 17:

6.1 Document **D2** discloses:
A method for culturing (page 8, line 61: "Kultivieren... von ... Zellen") anchorage dependent and non-anchorage dependent (This requirement is met by the disclosure of D2 since the feature "anchorage dependent and non-anchorage dependent" covers all possible kinds of cells. Thus, this feature has no limiting

effect.) cells (page 8, line 42: "Zellkulturen oder Mikroorganismen") that in a part of their life cycle require attachment to a substrate surface (page 3, line 61: "...sich die Zellen auf den Hohlfasern absetzen"; page 8, line 66: "...Zellen und Enzyme auf der Oberfläche der Hohlfasern immobilisiert werden") where the substrate surface is made in a plastic material (claims 4 and 5) and has an immediate water contact angle ≤ 30° (page 8, line 28).
Therefore, the subject-matter of independent **claim 17 is not new versus D2** (Art. 33(2) PCT).

6.2 Dependent **claims 18 and 22** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty, the reasons being as follows:

6.2.1 The additional features of dependent **claim 18** have already been disclosed in D2 (see claim 1).

6.2.2 The additional features of dependent **claim 22** have already been disclosed in D2 (see figures): The device ("Trennmodul") disclosed in figures 1 and 2 is considered "micro-fabricated" because it contains micro-porous capillary fibres (see claim 1) with a sub-micron diameter (page 9, line 64: "Innendurchmesser: 240 µm"). Cells are cultivated on the outer surface of the fibres (page 8, line 66) which are inside the "Trennmodul". Therefore, cell culturing is carried out "in a chamber of a microfabricated device". The fibres function as channels which supply the cells with serum (page 8, line 46). Due to their inner diameter of 240 µm, the fibres meet the requirement that the "channel having a depth which is ≤ 1000 µm".

6.3 Dependent claims 19 to 22 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:

6.3.1 Concerning dependent **claim 19**, D2 already discloses for anchorage dependent cells that the cells adhere to the substrate surface (page 8, lines 45 and 66). It is therefore considered an obvious variation falling within the scope of the man skilled in the art to make sure that at least 30% of these

cells adhere.

6.3.2 The additional features of dependent **claims 20 and 21** relate to well known practices in the art of culturing cells and are therefore considered obvious for the person skilled in the art.

Re Item VII

Certain defects in the international application

7. The document DE 3712491 is incorrectly described on amended page 2 of the description (lines 30 and 31), since that document explicitly mentions plastic material (see claims 4 and 5).

is attributed to degradation of polymer chains during the plasma treatment.

Similar effects have also been observed for polystyrene. An ESCA-study of a plasma-treated tissue-culture polystyrene showed about 35% loss of surface oxygen after water washing (Onyiriuka et al., J. Coll. Interf. Sci. 144(1) (1991) 98). In two other ESCA studies, oxygen-plasma treated polystyrene gave 25% surface oxygen loss after water washing (Callen et al., J. Vac. Sci. Technol. A 13(4) (1991) 2023-2029), (Morra et al., Angew. Macromol. Chem. 189 (3184) (1991) 125-136). A polystyrene surface treated with an oxygen plasma had initially a water-contact angle of 7°, but after a methanol wash the contact angle increased to 64° (Murakami et al., J. Coll. Interf. Sci. 202 (1998) 37-44).

WO 9618498 describes an attempt to produce a permanently hydrophilised surface made of plastics. The method comprises a first step in an inorganic gas plasma in order to introduce charges on the surface and a second step during which a polyionic polymer having the opposite charge is adsorbed to the surface.

EP-A-106,046 describes hydrophilisation of fluorinated polymer surfaces by treatment in a gas plasma in which the main component is a polymerising nitrogen-containing organic compound.

GB 2,061,969 describes the manufacture of hydrophilic and antistatic vinyl chloride polymer by treatment in an inorganic gas plasma. The problem of rendering the plastics permanently hydrophilic is not mentioned.

DE 3712491 describes gas plasma hydrophilization of porous membranes made of various synthetic polymers. Plastic material is not mentioned. Storage stability of the hydrophilized membranes is indicated but there is no discussion related to stability during washing conditions in aqueous milieu. Liquid contact angles are measured, but since

porous surfaces typically gives significantly decreased angles compared to no-porous smooth surfaces, these values cannot simply be compared with the values given in this specification.

EP 106662 describes a microtiter plate made of a dark plastic material in order to improve fluorescence measurements in the wells. Microtiter plates are normally devoid of liquid transportation systems in microformat.

The electric excitation field applied typically has a frequency in the radiowave or microwave region, i.e. kHz-MHz or GHz respectively. The modification (hydrophilisation) on the polymer surface caused by the plasma will depend mainly on a number of internal plasma parameters such as: type of species present in the plasma, spatial distributions, energy distributions and directional distributions. In turn these parameters depend in a complex way on the external plasma parameters: reactor geometry, type of excitation, applied power, type of process gas, gas pressure and gas flow rate.

In many applications involving contact between polar liquids and surfaces it is of no big concern whether an introduced hydrophilicity is stable towards washing or not. Particular problems are encountered in case the polymer surface is part of a channel of capillary dimensions, where a high degree of hydrophilicity is necessary if aqueous liquids are to be introduced by self-suction or by centripetal forces. This becomes particularly true in case a repeated contact is to take place reproducibly, in which case an unstable surface modification will be washed away during the first liquid contact. The smaller dimensions of the channel the more severe the problem becomes.

In the context of the invention the expression "plasma treated surface" will, if not otherwise specified, refer to an uncoated naked plasma treated surface, possibly being

derivatized to contain separate reactive species firmly bound to the surface.

Cell culturing in microfabricated devices has been described previously in for instance WO 9955827 with priority from April 27, 1998.

WO 9721090 relates to a microfluidic device in which centrifugal force is used to drive the liquids. In one sentence it is suggested that microculture and identification of pathogens can be made within the device without any hint at the selection of the proper surface characteristics.

Cell aggregates have previously been cultured in vessels with a water contact angle below 30° (JP patent application 19930119579, Derwent abstract accession number 1995-047885.

Adherence of cells to gas plasma treated polytetrafluoro ethylene (PTFE) surfaces with water contact angles 20-45° has been studied (Dekker et al., Clinical Materials 11 (1992) 157-162). Adherence appears to have required abnormally high concentrations of substances promoting adherence (20 % human serum-containing culture medium) compared to the most commonly used 10 % or less.

Adherence of CHO cells to surfaces which have been gas plasma hydrophilised in the presence of H_2O-vapour has been studied in order to look for optimal cell cultivation properties of plastic surfaces (Lee et al., Biomaterials

C L A I M S

1. A method for rendering a substrate surface made in plastic material more hydrophilic by treatment in a gas plasma of a non-polymerizable gas, **characterized** in that the intensity of the plasma is selected so that the surface becomes permanently more hydrophilic, and with the optional step that the surface subsequently is washed with a pure solvent selected from water, a water-miscible solvent and mixtures thereof,
said surface containing a liquid transportation system in which there is a channel with a depth $\leq 1000\mu m$.

2. The method according to claim 1, **characterized** in that the plasma intensity is ≥ 5 W/cm^3/min.

3. The method according to claim 2, **characterized** in that a power ≥ 250 W and a gas flow ≤ 50 cm^3/min are applied.

4. The method of any one of claims 1-3, **characterized** in that the plastic material has been selected among plastics having an immediate water-contact angle $\geq 20°$ and
(a) the plasma treatment conditions are set so that the immediate water-contact angle after plasma treatment and a subsequent wash with pure water/ethanol (70 % w/w) becomes $\leq 30°$.

5. The method of any one of claims 1-4, **characterized** in that the plastic material is selected among
(a) polymers/copolymers in which the monomers are unsaturated such as (i) alkenes/alkadienes, and acids, esters, amides, nitriles etc containing one or more alkene groups; and (ii) vinyl aryl compounds in which the vinyl group is bound to aryl that optionally is substituted with lower alkyl groups (C1-6);
(b) condensation polymers/copolymers in which the monomomers are selected from compounds exhibiting two

or more groups selected among amino, hydroxy, carboxy etc groups.

6. The method of any one of claims 1-5, **characterized** in that the plasma is induced by radio- and/or microwaves.

7. The method of any one of claims 1-6, **characterized** in that the plasma gas is oxygen, nitrogen, or a noble gas, such as argon, or a mixture of these gases.

8. The method of any one of claims 1-7, **characterized** in that subsequent to the plasma treatment the surface of the substrate is derivatized to exhibit groups selected amongst anion exchanging groups, cation exchanging groups, amphoteric groups, hydroxy groups, bioaffinity groups, and chelating groups.

9. The method of any one of claims 1-8, **characterized** in that at least part of the surface comprises a liquid transportation system comprising a channel which has a depth $\leq$ 1000 μm.

10. A substrate surface which is made of a plastic material,which has been plasma treated, **characterized** in that the surface in uncoated form has an immediate water-contact angle $\leq$ 30°, said water-contact angle being changed less than ± 20% and/or less than ± 5° upon washing with a pure ethanol/water mixture (70% w/w).

11. The substrate of claim 10, **characterized** in that the plastic material is selected from

(a) polymers/copolymers in which the monomers are unsaturated such as (i) alkenes/alkadienes , and acids, esters, amides, nitriles etc containing one or more alkene groups; and (ii) vinyl aryl compounds that optionally is substituted with lower alkyl groups (C1-6);

(b) condensation polymers/copolymers in which the monomomers are selected from compounds exhibiting two or more groups selected among amino, hydroxy, carboxy etc groups;

said polymer material optionally being cross-linked and/or optionally being a mixture of two or more kinds of polymers/copolymers.

12. The substrate of any one of claims 10-11, **characterized** in that the surface before having been gas plasma treated exhibits an immediate water-contact angel $\geq 30°$.

13. The substrate of any one of claims 10-12, **characterized** in that at least part of the surface comprises a liquid transportation system comprising a channel which has a depth ≤ 1000 μm.

14. The use of the substrate defined in any one of claims 10-13 and/or obtained by the method defined in any one of claims 1-9 for the culturing of cells.

15. A kit **characterized** in comprising

(a) a microfabricated device comprising a substrate surface which is made in a plastic material and which comprises a liquid transportation system comprising a channel which has a depth ≤ 1000 μm, and

(b) a fluorescent substance to be detected in the device, the fluorescence intensity of the plastic material being < 50 % of the fluorescent intensity of the substance at the wavelength at which the substance fluoresces.

16. The kit of claim 15, **characterized** in that the plastic comprises a polymerisation product obtained by polymerisation of an aliphatic monomer in which there is unsaturation.

17. A method for culturing anchorage-dependent cells and non-anchorage dependent cells that in a part of their life cycle require attachment to a substrate surface, **characterized** in that the substrate surface is made in a plastic material and hasan immediate water-contact angle $\leq 30°$.

18. The method of claim 17, **characterized** in that the substrate surface has been obtained by the gas plasma treatment method defined in claims 1-9.

19. The method of any one of claims 17-18, with the proviso that for anchorage-dependent cells the substrate surface enables at least 30 % of the plated cells to adhere to the substrate surface.

20. The method of any one of claims 17-19, **characterized** in that at most 15 % of the culture medium is serum.

21. The method of any one of claims 17-20, **characterized** in that culturing is taking place during a time period permitting the number of cells to be at least duplicated.

22. The method of any one of claims 17-21, **characterized** in that the cell culturing is carried out in a chamber of a microfabricated device in which there is a liquid transportation system comprising (a) a channel having a depth which is ≤ 1000 μm and (b) said chamber which also provides said substrate surface.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PU9909-PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/02632	24/03/2000	24/03/1999
International Patent Classification (IPC) or national classification and IPC C08J7/00		
Applicant GYROS AB		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 12 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/10/2000	04.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Kujat, C Telephone No. +49 89 2399 2360

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/02632

I. Basis of the report

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   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,3,8,10-14,19-21
	No:	Claims	1,4,-7,9,15-18,22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	2,3,8,10-14,19-21
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations
   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item VIII

Certain observations on the international application

1.1 **Independent claim 1** lacks clarity (Art. 6 PCT) for the following reasons:

1.1.1 The feature "said surface containing a liquid transportation system in which there is a channel with a depth ≤ 1000 µm" in the method claim 1 relates to characteristics of a substrate surface rather than clearly defining the method in terms of its method steps. The intended limitations and the category of the claim are therefore not clear from this claim, contrary to the requirements of Article 6 PCT.

Since claim 1 appears to be directed to the application of a "method for rendering..." to a specific product ("a surface containing a liquid transportation system..."), claim 1 will be construed during the subsequent assessment of novelty and inventive step having the meaning:

A method for rendering a substrate surface made in plastic material more hydrophilic, **said surface containing a liquid transportation system in which there is a channel with a depth ≤ 1000 µm**, by treatment in a gas plasma... .

1.1.2 The feature "the intensity of the plasma is selected so that ... more hydrophilic" does not meet the requirements of article 6 PCT because it attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It is possible to define the invention in more concrete terms, see e.g. the values given in dependent claims 2 and 3.

1.1.3 Moreover, the optional feature "the surface subsequently is washed with a pure solvent selected from water, a water-miscible solvent and mixtures thereof" does not meet the requirements of article 6 PCT because it contains a contradiction: A mixture of water and a water-miscible solvent cannot be a "pure" solvent.

1.1.4 Further, the feature "permanently more hydrophilic" does not meet the requirements of article 6 PCT because it is not clear by which means "permanently more hydrophilic" differs from "more hydrophilic". A test

procedure defining criteria which enable the skilled man to distinguish a "permanently more hydrophilic" surface from a "more hydrophilic" surface would meet the criteria of Art. 6 PCT.

1.2 The additional feature of dependent **claim 4**, "conditions are set so that ..." does not meet the requirements of article 6 PCT because it is not clear which conditions, e.g. plasma intensity, frequency, power or flow of gas supply, are referred to.
Moreover, the feature "conditions are set so that ..." also attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It appears possible to define the invention in more concrete terms, see e.g. the values given in dependent claim 2. This feature is unclear as it appears to be the result of plasma treatment conditions which are not further defined.

1.3 The feature of **independent claim 14**, "the culturing of cells" does not meet the requirements of article 6 PCT because it is not clear which scope of protection the term "culturing" shall define. A proper definition, e.g. as given on page 16 of the description would render claim 14 clear.

1.4 The feature "in uncoated form" in **independent claim 10** does not meet the requirements of Art. 6 PCT, since it is not clear whether "uncoated" refers to the state of the surface before or after plasma treatment. Since the plasma treatment in the application in suit is performed with a non-polymerizable gas (see independent claim 1), no coating will be applied during the plasma treatment. "Uncoated" is therefore construed as having the meaning "immediately after plasma treatment".

1.5 The feature of **dependent claim 19**, "the substrate surface enables at least 30% of the plated cells to adhere" does not meet the requirements of article 6 PCT because it attempts to define the invention by a result to be achieved (PCT-Gazette, section IV, chapter III-4.7). It appears possible to define the invention in more concrete terms, e.g. in terms of how the surface has been obtained or which structure/texture it has.

1.6 The additional feature "micro fabricated device" in dependent **claim 22** does not

meet the requirements of article 6 PCT because it is not clear in how far a micro fabricated device differs from a conventionally fabricated device.
It would be clear to add the definition given on page 12, lines 3 to 6, to said claims.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Not-withstanding the clarity objections raised under Item VIII, novelty and inventive step will now be assessed. Reference is made to the following documents:

D1: WO 97/21090
D2: DE 3712491
D3: EP 467639

Independent Claim 1:

2.1 Document **D1** discloses a substrate surface (pages 17 and 19: injection moulded plastic disc) which contains a liquid transportation system (page 1, line 17: "utilizing the centripetal forces resulting from rotation of the platform to motivate fluid movement") in which there is a channel (page 1, line 18: "fluid movement through micro channels") with a depth ≤ 1000 µm (page 12, line 6: "is less than 500 µm").
D1 further discloses that there is a strong correlation between water contact angle and cell adsorption, with hydrophilic surfaces showing significantly less cell adsorption than hydrophobic surfaces (page 20, lines 9 and 10) and that it is desirable to increase the hydrophilicity of such a surface (page 20, line 14). For doing so, D1 suggests the use of inert gas plasmas (page 20, line 32).

Due to the disclosure of such a substrate in D1, a "method for rendering a substrate surface made in plastic material more hydrophilic by treatment in a gas plasma of a non-polymerizable gas" is also disclosed by D1.

Further, the skilled person is well aware that he must precisely select certain parameters of the plasma (e.g. intensity, power, frequency, gas flow-rate) during plasma treatment in order to reproducibly obtain a desired result.

2.2 Therefore, the subject-matter of independent **claim 1 is not new versus D1** (Art. 33(2) PCT).

2.3 Dependent **claims 2, 3 to 8** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of **inventive step**, the reasons being as follows:

2.3.1 The additional features of dependent **claims 2 and 3** appear to relate to common parameters in plasma treatment which appear to be obvious for the skilled person. See e.g. **D2** (plasma intensity of greater than 5 W/cm^3/min (page 9, lines 20 to 27: 60 W divided by 8.5 sccm equals 7.0588 W/cm^3/min)) or **D3** discloses a power ≥ 250 W (page 5, line 3).

2.3.2 Concerning the additional features of dependent **claim 8,** the derivatization of the surface appears to be a common practice in the field of plasma treatment, which is therefore devoid of inventive activity.

2.4 Dependent **claims 4, 5, 9** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of **novelty**, the reasons being as follows:

2.4.1 Apart from the disclosure of materials in **D2** (page 2, line 13 "polyolefin", which includes both polypropylene and polyethylene. Both materials have an immediate water-contact angle of greater than 90°, see description, page 8. The immediate water-contact angle after treatment is down to 21°, page 8, line 28), the materials of dependent **claims 4 and 5** have also been disclosed in **D1** (page 17, last paragraph). The feature "(a) the plasma treatment conditions are set so that..." is not discussed as it is not clear (see paragraph 1.2).

2.4.2 The additional features of dependent **claims 6 and 7** have been implicitly

disclosed in **D1** (page 20, line 32): The skilled person in the field of plasma treatment knows well that an inert-gas (or reactive-gas) plasma is induced by a frequency corresponding to radio- or microwaves. He further knows well that the plasma gas is nitrogen or a noble gas for inert-gas plasmas.

2.4.3 The additional feature of dependent **claim 9** has already been disclosed in document **D1** (page 12, line 6: "is less than 500 µm").

Independent Claim 10:

3.1 Document **D2** discloses a substrate surface (surface of the membrane "Mikrofiltrations-Membran", see claim 1) which is made of a plastic material (see claims 4 and 5), which has been plasma treated (see claim 1: "Behandlung mit einem nicht-polymerisierbaren Plasmagas"), where the surface in uncoated form (i.e. directly after plasma-treatment) has an immediate water contact angle ≤ 30° (page 8, line 28).

3.2 The subject-matter of independent claim 10 differs from the disclosure of document **D2** in that the water-contact angle (is) changed less than ± 20% upon washing with ethanol/water mixture (70% w/w). (The feature "a pure ethanol/water mixture" lacks clarity for the reasons given in paragraph 1.1.3).

3.3 This feature per se is unclear for the reasons given in paragraph 1.2 as it appears to be the result of plasma treatment conditions which are not further defined. Not withstanding this objection, the inventive step will now be discussed:
Such a characteristic seems to be implicitly disclosed in D2, because D2 highlights the low contact angle (page 9, lines 30 and 31: "Benetzbarkeiten ... haben sich bemerkenswert verbessert") for wafer-washing-solution (page 9, line 30: "Wafer Wasch/Kühlmittel ... Aqua Kool 7X").
Both the ethanol/water mixture and the wafer-washing solution of D2 are considered solvents.
Moreover, the feature "washing" does not seem to have any limiting effect further than the meaning "bringing into contact with". Since the membrane of **D2** is immersed in a liquid (see e.g. claims 27 and 28) during its intended use, it is assumed that "washing" takes place during that use. The information relating to

the wettability ("Benetzbarkeit") on page 9, line 30 must therefore be interpreted in the light of this intended use.

3.4 Therefore, the disclosure of an equivalent substance in **claim 10 is not considered involving an inventive step** (Art. 33(3) PCT).

3.5 Dependent **claims 11 to 13** do not appear to contain any additional features, which in combination with the features of any claim to which they refer meet the requirements of the PCT with regard to inventive step, the reasons being as follows, and the problem-solution-approach-analysis remaining the same, the distinguishing features over D1 remaining identical:

3.5.1 The additional features of dependent **claim 11** have already been disclosed in D2 (page 2, line 14).

3.5.2 The additional features of dependent **claim 12** have already been disclosed in D2 (page 2, line 13 "polyolefin", which includes both polypropylene and polyethylene. Both materials have an immediate water-contact angle of greater than 90°, see description, page 8.

3.5.3 The additional features of dependent **claim 13** render independent claim 10 unclear. It is not possible to say whether the substrate surface disclosed in **D1** meets the requirement relating to the immediate water contact angle defined in claim 10 (thus, destroying novelty of claim 13).

Independent Claim 14:

4. Independent claim 14, which relates to the use of the product obtained by any of claims 1 to 13, can not be considered involving an inventive step (Art. 33(3)) for the lack of clarity explained in paragraph 1.3.

Independent Claim 15:

5.1 Concerning independent claim 15, document **D1** discloses a kit comprising a micro fabricated device (page 5, line 24: "micro platform disk") comprising a substrate surface which is made in a plastic material (page 17, lines 30 to 34) and which comprises a liquid transportation system (page 1, line 17: "utilizing the centripetal forces resulting from rotation of the platform to motivate fluid movement") comprising a channel (page 1, line 18: "fluid movement through micro channels") which has a depth ≤ 1000 µm (page 12, line 6: "is less than 500 µm") and a fluorescent substance * to be detected in the device.

* Since the device disclosed in **D1** contains fluorescent detecting means (page 11, line 5) and since D1 relates to fluorescence excitation (page 31, line 2), a fluorescent substance is used as a fluid in a corresponding application of the micro fabricated device of D1.

5.2 The additional feature "the fluorescence intensity of the plastic material being < 50% of the fluorescent intensity of the substance at the wavelength at which the substance fluoresces is considered an implicit feature of the micro fabricated device of D1, since it would not work otherwise.

5.3 Therefore, the subject-matter of independent **claim 15 is not new** versus **D1** (Art. 33(2) PCT).

5.4 The additional features of dependent **claim 16** have already been disclosed in **D1** (see page 17, lines 30 to 34).

Independent Claim 17:

6.1 Document **D2** discloses:
A method for culturing (page 8, line 61: "Kultivieren... von ... Zellen") anchorage dependent and non-anchorage dependent (This requirement is met by the disclosure of D2 since the feature "anchorage dependent and non-anchorage dependent" covers all possible kinds of cells. Thus, this feature has no limiting

effect.) cells (page 8, line 42: "Zellkulturen oder Mikroorganismen") that in a part of their life cycle require attachment to a substrate surface (page 3, line 61: "...sich die Zellen auf den Hohlfasern absetzen"; page 8, line 66: "...Zellen und Enzyme auf der Oberfläche der Hohlfasern immobilisiert werden") where the substrate surface is made in a plastic material (claims 4 and 5) and has an immediate water contact angle ≤ 30° (page 8, line 28).
Therefore, the subject-matter of independent **claim 17 is not new versus D2** (Art. 33(2) PCT).

6.2 Dependent **claims 18 and 22** do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty, the reasons being as follows:

6.2.1 The additional features of dependent **claim 18** have already been disclosed in D2 (see claim 1).

6.2.2 The additional features of dependent **claim 22** have already been disclosed in D2 (see figures): The device ("Trennmodul") disclosed in figures 1 and 2 is considered "micro-fabricated" because it contains micro-porous capillary fibres (see claim 1) with a sub-micron diameter (page 9, line 64: "Innendurchmesser: 240 µm"). Cells are cultivated on the outer surface of the fibres (page 8, line 66) which are inside the "Trennmodul". Therefore, cell culturing is carried out "in a chamber of a microfabricated device". The fibres function as channels which supply the cells with serum (page 8, line 46). Due to their inner diameter of 240 µm, the fibres meet the requirement that the "channel having a depth which is ≤ 1000 µm".

6.3 Dependent claims 19 to 22 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, the reasons being as follows:

6.3.1 Concerning dependent **claim 19**, D2 already discloses for anchorage dependent cells that the cells adhere to the substrate surface (page 8, lines 45 and 66). It is therefore considered an obvious variation falling within the scope of the man skilled in the art to make sure that at least 30% of these

cells adhere.

6.3.2 The additional features of dependent **claims 20 and 21** relate to well known practices in the art of culturing cells and are therefore considered obvious for the person skilled in the art.

Re Item VII

Certain defects in the international application

7. The document DE 3712491 is incorrectly described on amended page 2 of the description (lines 30 and 31), since that document explicitly mentions plastic material (see claims 4 and 5).

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(30) Priority Data:
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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

(88) Date of publication of the international search report:
22 March 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/56808 A3

(54) Title: SURFACE AND ITS MANUFACTURE AND USES

(57) Abstract: A method for rendering a surface covered by a polymer material (plastics) more hydrophilic by treatment in a gas plasma of a non-polymerizable gas. The method is characterized in that the intensity of the plasma is selected so that the surface becomes permanently more hydrophilic. A naked plasma treated surface of plastics having an immediate water-contact angle ≤ 30°, such as ≤ 20°, said water-contact angle being changed less than ± 20 % and/or less than ± 5° upon washing with ethanol/water mixture (70 % w/w). A kit comprising (a) a microfabricated device comprising a surface (i) which is manufactured from a synthetic polymer material (plastics) and (ii) on which there are at least one chamber and/or at least one channel, and (b) a fluorescent substance to be detected in the device. The kit is characterized in that the plastics has a fluorescence that is non-significant with respect to the fluorescence of the substance at the wavelength at which the substance fluoresces. A microfabricated device having a liquid transportation system which is formed in/on a polymer material (plastics) that is a polymerised aliphatic monomer containing unsaturation.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 00/02632

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B29C59/14 C12N5/00 B01L3/00 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B29C C12N B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	DE 37 12 491 A (APPLIED MEMBRANE TECH) 15 October 1987 (1987-10-15) page 4, line 67 -page 5, line 26 page 7, line 40 -page 8, line 3 page 8, line 40 -page 9, line 2 page 9, line 12 - line 60; claims; figures ---	1-8, 15-17,21 9-13
X Y	EP 0 467 639 A (ITOH C FINE CHEMICAL CO ;EC CHEM IND CO (JP); KOGOMA MASUHIRO (JP)) 22 January 1992 (1992-01-22) page 4, line 53 -page 5, line 5 page 5, line 10 - line 27; claims; figures ---	1,2,4-6 9-13
X	US 4 741 619 A (HUMPHRIES GILLIAN M K ET AL) 3 May 1988 (1988-05-03) the whole document --- -/--	1,3-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2001	22. 01. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Labeeuw, R

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/02632

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 111 795 A (NIKKA CHEMICAL IND CO LTD ;EMORI & CO LTD (JP); SHINETSU CHEMICAL) 27 June 1984 (1984-06-27) page 4, line 27 -page 5, line 6 page 6, line 13 - line 21 page 8, line 16 -page 9, line 10 ---	1,2,5-7
X	WO 97 21090 A (GAMERA BIOSCIENCE) 12 June 1997 (1997-06-12)	15-21
A	page 19, line 8 -page 20, line 10; figures ---	1-13
P,X	WO 99 58245 A (AMERSHAM PHARM BIOTECH AB ;ALLMER KLAS (SE); ANDERSSON PER (SE); L) 18 November 1999 (1999-11-18) cited in the application page 2, line 14 - line 21; claims 1-4,11,12; figures; example 1 ---	1-4, 8-13, 15-17, 20,21
A	GB 2 061 969 A (SHINETSU CHEMICAL CO) 20 May 1981 (1981-05-20) cited in the application the whole document ---	1,9
X	EP 0 106 662 A (DYNATECH LAB) 25 April 1984 (1984-04-25) the whole document ---	14
X	DE 197 39 119 A (UNIV SCHILLER JENA) 11 March 1999 (1999-03-11) column 2, line 1 - line 18; claim 1; figures ---	14
P,X	US 6 027 695 A (OLDENBURG KEVIN R ET AL) 22 February 2000 (2000-02-22) the whole document and in particular column 1 line 36 - line 61, column 3 line 4 - line 23 and column 7 line 66 - column 8 line 29 ---	14
A	US 5 399 316 A (YAMADA TAKASHI) 21 March 1995 (1995-03-21) column 4, line 60 -column 5, line 12 column 5, line 43 - line 53; figure -----	14

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/02632

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-8

   Making a plastics surface permanently more hydrophilic by the selected intensity of a gas plasma treatment

   1.1. Claims: 9-13
   A plasma treated hydrophilic surface whose water-contact angle changes less than 20% upon washing

   1.2. Claims: 15-20
   A hydrophilic plastics surface used for culturing cells by their attachment

   1.3. Claim : 21
   A microfabricated liquid transportation system

2. Claim : 14

   A microfabricated device with a plastics surface having a fluorescence intensity less than 50% of the fluorescence intensity of a substance detected in the device

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern al Application No
PCT/EP 00/02632

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3712491 A	15-10-1987	AU 7135087 A	15-10-1987
		DK 185787 A	12-10-1987
		JP 62294407 A	21-12-1987
EP 0467639 A	22-01-1992	JP 3063769 B	12-07-2000
		JP 4074525 A	09-03-1992
		CA 2047147 A	18-01-1992
		DE 69128502 D	05-02-1998
		DE 69128502 T	23-07-1998
		KR 169490 B	20-03-1999
		US 5124173 A	23-06-1992
US 4741619 A	03-05-1988	EP 0290125 A	09-11-1988
EP 0111795 A	27-06-1984	JP 59106567 A	20-06-1984
		JP 59106568 A	20-06-1984
		DE 3377148 D	28-07-1988
WO 9721090 A	12-06-1997	AU 702403 B	18-02-1999
		AU 1283397 A	27-06-1997
		CA 2239613 A	12-06-1997
		CN 1208464 A	17-02-1999
		EP 0865606 A	23-09-1998
		NO 982563 A	05-08-1998
		AU 4144897 A	06-03-1998
		EP 0917648 A	26-05-1999
		WO 9807019 A	19-02-1998
		US 6143248 A	07-11-2000
WO 9958245 A	18-11-1999	AU 3624399 A	29-11-1999
		GB 2350678 A	06-12-2000
		GB 2341924 A	29-03-2000
GB 2061969 A	20-05-1981	JP 1338119 C	29-09-1986
		JP 56061434 A	26-05-1981
		JP 60039290 B	05-09-1985
		DE 3039852 A	17-09-1981
		FR 2467865 A	30-04-1981
		NL 8005814 A	27-04-1981
EP 0106662 A	25-04-1984	AU 564381 B	13-08-1987
		AU 1929583 A	19-04-1984
		DE 3378545 D	29-12-1988
		ES 525541 D	16-12-1984
		ES 8501998 A	16-03-1985
		ES 535979 D	01-03-1987
		ES 8703753 A	16-05-1987
		JP 2078438 C	09-08-1996
		JP 3040818 B	20-06-1991
		JP 59132335 A	30-07-1984
		US 4725388 A	16-02-1988
DE 19739119 A	11-03-1999	NONE	
US 6027695 A	22-02-2000	NONE	
US 5399316 A	21-03-1995	JP 5253498 A	05-10-1993
		DE 4307735 A	23-09-1993